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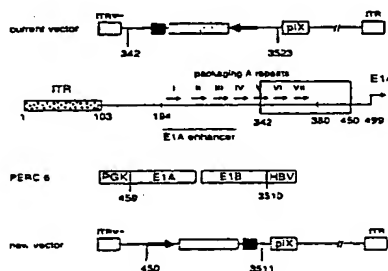
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(54) Title: **ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS**



Modifications made to the current adenovector backbone in the generation of the new vector.

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S.
provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2
(serial number unassigned), filed September 15, 2000, March 27, 2001, and
September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first
generation adenovirus vaccines found to exhibit enhanced growth properties and
20 greater cellular-mediated immunity as compared to other replication-deficient vectors.
The invention also relates to the associated first generation adenoviral vectors
described herein, which, through the incorporation of additional 5' adenovirus
sequence, enhance large scale production efficiency of the recombinant, replication-
defective adenovirus described herein. Another aspect of the instant invention is the
25 surprising discovery that the intron A portion of the human cytomegalovirus (hCMV)
promoter constitutes a region of instability in adenoviral vector constructs. Removal
of this region from adenoviral expression constructs results in greatly improved vector
stability. Therefore, improved vectors expressing a transgene under the control of an
intron A-deleted CMV promoter constitute a further aspect of this invention. These
30 adenoviral vectors are useful for generating recombinant adenovirus vaccines against
human immunodeficiency virus (HIV). In particular, the first generation adenovirus
vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-
1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide
pharmaceutical products, and biologically active modifications thereof. Host
35 administration of the recombinant, replication-deficient adenovirus vaccines described
herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5 The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

10 The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus
15 to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes
20 while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where
25 the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus
30 (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to
35 day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0
5 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region
10 are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of
15 incorporated individual A (packaging) repeats; see, e.g., Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction
20 with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results
25 in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several
30 mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral
35 replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5' region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in
15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use
20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

- 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene
10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

- Other aspects of this invention include a host cell comprising said adenoviral
15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

- To this end, the present invention particularly relates to harvested
20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6[®] cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material
25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

- Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual
30 an adenovirus vaccine vector comprising:

- a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,
35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5 The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not
10 limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen
15 with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of
20 such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

 The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be
25 ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)
30 within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second
35 harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair
20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a
25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV
30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a
35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

35 "Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+bGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*III site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene in the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises
 10 codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns
 15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as
 20 "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

35 Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5 Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20 Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5 Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed
10 herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences
15 through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate
20 consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding
25 sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as
30 underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino
acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174
35 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5 Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10 Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15 Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20 Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25 Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30 Figure 31 shows the intracellular γ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- γ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ γ IFN+ and CD4+ γ IFN+, respectively.

35 Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6[®] cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include
5 the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and
10 MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24,
15 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and
20 Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene
25 closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale.
30 As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g.,
35 mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon
5 optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 *pol* open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with
5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral
10 composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a
15 combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

20 Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.
25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino
30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most
35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells
5 for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully
10 transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of
15 this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

20 Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient
25 to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed
30 *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin
35 resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene
20 product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVII_{ns}HIVgag was used as the starting material to amplify the hCMV promoter. PVI_{ns}HIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *Bgl*III recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *Bgl*III. This fragment was then cloned back into the original GMP grade pVI_{ns}HIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *Bgl*III digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pVI_{ns}HIVgag vector backbone. This vector is designated pVII_{ns}CMV(no intron).

The FLgag gene was excised from pVI_{ns}HIVgag using *Bgl*III digestion and the 1,526 bp gene was gel purified and cloned into pVI_{ns}CMV(no intron) at the *Bgl*III site. Colonies were screened using *Sma*I restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pVI_{ns}CMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	$\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$
HIVFL-gagPR9901 ^a	10.8
PV1Jns-hCMV-FLgag-bGHpA ^b	16.6
pV1Jns-hCMV-FLgag-SPA ^{b,c}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

5 ^b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

10

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above
 15 possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
 20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20 μg and 200 μg .

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA ^a Promoter/terminator	Dose, ug ^b	Anti-p24 Titers (3 Wk PD1) ^c			SFC/10 ⁶ Cells (4 Wk PD1) ^d		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV- FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV- FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

^ain PBS^bi.m. Injections into both quads, 50 µL per quad^cn=10; GMT, geometric mean titer; SE, standard. error^dn=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

- The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:
- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
 - (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).
- These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

- Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with *Pac1* and *BstZ1101* and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla1* linearized pAdHVO (E3- adenovector) or *Cla1* linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *ClaI* , *BamHI*, *Xho I*, *EcoRV*, *HindIII*, *Sal I*, and *Bgl II* sites. This MCS was replaced with a new MCS containing *Not I*, *Cla I*, *EcoRV* and *Asc I* sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested.

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and then labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

5 Construction of the new shuttle vector containing modified gag transgene –
“MRKpdelE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeIE1 shuttle vector.

EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*I. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*I overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH₂O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bsr*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1 gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac1* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6[®] cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6[®] cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [³³P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac1/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5 Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10 Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10 ⁶ cells/ml), Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁶ vp/ml culture	Titer 10 ⁶ vp/cell	QPA 10 ⁶ TCID ₅₀ /ml	Ratio AEX/QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 69%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 94%	0.85, 64%	47.5	54	9.0	8.7	1.10	82	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4.4	4.9	1.03	43	175	3.12
P10	1.09, 91%	1.06, 66%	47.5	58	3.0	2.8	1.16	26	100	2.84
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	2.85
P14	1.94, 82%	0.88, 67%	46	53	6.6	4.4			160	2.60
P15	0.97, 96%	0.64, 66%	47	47	8.9	7.1			250	3.18
										3.28
										3.27
										3.12
										2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10 ⁶ cells/ml), Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁶ vp/ml culture	Titer 10 ⁶ vp/cell	QPA 10 ⁶ TCID ₅₀ /ml	Ratio AEX/QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.82, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	170	
P6	1.55, 85%	1.26, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1.09, 87%	1.11, 81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 63%	48	56	2.1	2.1	0.47	45	75	3.12
P9	1.20, 89%	1.25, 81%	47.5	58	0.8	0.7	0.29	28	25	2.84
P10	0.99, 82%	1.55, 85%	47	60	2.3	2.3	0.43	53	80	2.70
P11	1.07, 96%	1.25, 63%	48	47	2.7	2.5	0.41	68	90	2.70
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	260	2.85
P13	1.98, 85%	1.14, 85%	45.5	53	5.8	3.0			110	2.60
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	9.7			350	3.18
P15	0.87, 99%	0.97, 69%	49.5	49	5.3	6.1			218	3.28
										3.27
										3.12
										2.91
										2.78
										2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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MRKAd5gag(E3-)

	Xv (10 ⁴ cells/ml), Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	QPA 10 ⁴ TCID ₅₀ /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 87%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	105	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.84
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	2.70
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	2.86
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.18
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	3.28
										3.27
										3.12
										2.91
										2.78
										2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10^7 and 10^9 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors ^a	$\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^e	0.42

^a $A_{260\text{nm}}$ absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

^d Research Ad5FLgag lot# 6399

^e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	^a MRKAd5gag	10 ⁷	25600	5877	4780
2		10 ⁹	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10 ⁷	7352	2077	1620
4		10 ⁹	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10 ⁷	12800	9905	236
6		10 ⁹	310419	99181	75165
7	^b mCMV FL-gag bGHpA [E3+] →	10 ⁷	44572	23504	15389
8		10 ⁹	941014	239068	190636
9	^c hCMV FL-gag bGHpA [E3-] ←	10 ⁷	3676	934	745
10		10 ⁹	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10 ⁶	528	262	175
12		10 ⁷	14703	5274	3882
13		10 ⁸	58813	14942	11915
14		10 ⁹	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10 ⁶	230	82	61
16		10 ⁷	4222	3405	1138
17		10 ⁸	19401	3939	3274
18		10 ⁹	89144	25187	19639
19	Naïve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal
P.I.s: Youil, Chen, Casimiro
Vaccination: T. Toner, Q. Su
Assay: M. Chen

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10¹¹ vp and 10⁹ vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood as summarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag ^a , 10 ¹¹ vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10 ⁹ vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag ^b , Clinical Lot, 10 ¹¹ vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10 ⁹ vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
^a MRKAd5gag (hCMV, bGHPA, E3+)								
^b original Ad5gag vector (hCMV/Intron A, bGHPA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp #	Vaccination T=0, 4, 25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media ^a	Gag H ^b	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag 10 ⁹ vp	97N010	6	89	0	395	0	1058	0	1174	3	775	4	1074
		97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	396	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAd5gag 10 ⁹ vp	97N120	5	275	1	249	4	141	4	119	9	206	4	219
		97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot 10 ⁹ vp	97X001	0	261	1	485	0	817	0	1220 ^b	1	894	0	1858
		97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot 10 ⁹ vp	97N020	3	30	1	101	0	66	0	36	0	26	0	41
		97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	85	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Native	96R041	6	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16	20	14	19	15	10	15	24	9

Based on either 4x10⁵ or 2x10⁶ cells per well (depending on spot density)

ND, not determined

^a mock or no peptide control

^b Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10⁹ vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

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EXAMPLE 17

CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

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The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCA GTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
 CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
 5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
 AACAAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
 CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
 GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
 10 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
 CCCGACAAGT GGA CTGTGCA GCCCATTTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
 15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
 CAGTGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
 20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
 GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
 25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
 ATCAGGAAGG TGCTGTTCTT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
 CACTCCAAC TGGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
 ATGTGTGGCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 30 TGCTCCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCTGTG TGAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGG GTCCATGAAC
 35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:2) .

The present invention especially relates to an adenoviral vector vaccine which
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to
 deletion of the portion of the wild type sequence encoding the protease activity, a
 30 combination of active site residue mutations are introduced which are deleterious to
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein
 the construct is devoid of DNA sequences encoding any PR activity, as well as
 containing a mutation(s) which at least partially, and preferably substantially,
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

```

5  AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
   ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
   GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
   GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
   CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
   TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
   AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
   CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
   GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
   ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
   CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
   GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
   ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
   GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
   CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
   ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
   GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
   TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
   GGGGCTGAGA CTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
   AAGAATGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
   GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
   GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
   GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
35 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
   CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

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ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 10 CAGATCACCA AGATCCAGAA CTTAGGGTG TACTACAGGG ACTCCAGGAA CCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACCTC
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
 NO:3).

15 In order to produce the IA-pol-based adenoviral vaccines of the present
 invention, inactivation of the enzymatic functions was achieved by replacing a total of
 nine active site residues from the enzyme subunits with alanine side-chains. As
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*
 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445,
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this
 IA Pol construct), with each residue being substituted for an Ala residue, respectively
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase
 function was abolished through three mutations at Asp626, Asp678 and Glu714.
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based
 adenoviral HIV vaccine of the present invention, either when administered alone or in
 a combined modality regime and/or a prime-boost regimen. For example, it may be
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,
 RNase-H, and integrase coding regions while still abolishing these enzymatic
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide
 such as is found in highly expressed mammalian proteins such as immunoglobulin
 leader peptides. Any functional leader peptide may be tested for efficacy. However,
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,
 preferably a leader peptide from human tPA. In other words, a codon optimized
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide
 at the amino terminal portion of the protein, which may effect cellular trafficking and
 hence, immunogenicity of the expressed protein within the host cell. As noted in
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
30 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCAT TGTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAAÇAGGG AGATCCTGAA
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGG CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGGAGC
 TGTGCAGAAG ATCACCAC TG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 15 GGAGGTGAAC ATTGTGACTG ACTCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 GTACCTGGCC TGGGTGCC TG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCAC TCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGACAATG GCTCCAAC TT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 25 GTGGGCTGTC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)
 which comprises a leader peptide at the amino terminal portion of the protein, which
 may effect cellular trafficking and hence, immunogenicity of the expressed protein
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,
 any such leader peptide-based HIV-1 pol mutant construct may include but is not
 limited to a mutated DNA molecule which effectively alters the catalytic activity of
 the RT, RNase and/or IN region of the expressed protein, resulting in at least
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at
 least one point mutation which alters the active site and catalytic activity within the
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
 CTTCTGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
 CCCCAGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT
 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCCTTAC
 CATCCCTTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
 GGGGTGGAAG GGCTCCCCTG CCATCTTCCA GTCTCCATG ACCAAGATCC TGGAGCCCTT
 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATG GTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGGAGGC
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 35 CCCTGAGTGG GAGTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCATG GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGTCTCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCAGTCCC AGGGGGTGGT
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 GAACCCCTG TGAAGGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfrl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

10 GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
15 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCCG CCCCCGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),
Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);
25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),
Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian
(human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby
incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.
codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating
methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides
660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid
HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine
vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID
35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions
 have been elucidated, it has become clear that correct trafficking of Nef to the inner
 plasma membrane promotes viral replication by altering the host intracellular
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the
 20 infectivity of progeny viral particles. In one aspect of the invention regarding
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the
 adenovirus vector of the present invention is modified to contain a nucleotide
 sequence which encodes a heterologous leader peptide such that the amino terminal
 region of the expressed protein will contain the leader peptide. The diversity of
 25 function that typifies eukaryotic cells depends upon the structural differentiation of
 their membrane boundaries. To generate and maintain these structures, proteins must
 be transported from their site of synthesis in the endoplasmic reticulum to
 predetermined destinations throughout the cell. This requires that the trafficking
 proteins display sorting signals that are recognized by the molecular machinery
 30 responsible for route selection located at the access points to the main trafficking
 pathways. Sorting decisions for most proteins need to be made only once as they
 traverse their biosynthetic pathways since their final destination, the cellular location
 at which they perform their function, becomes their permanent residence.
 Maintenance of intracellular integrity depends in part on the selective sorting and
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

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CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCGAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG
GCCCAGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCATGTG
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTTC ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jf1) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).
Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for
25 expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jf1 nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13,
35 as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
 ACACCCCCGG CCCCGGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACGTC GCCGCCACCC
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

30 An additional embodiment of the present invention relates to another DNA
 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue
 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
 TTCGCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
 GCCCAGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TCGCCGCCC ACCCCATGTC
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCT ACTCCAAGCT
 GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCCC
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,

regardless of codon usage, which expresses a wild type or modified Nef protein as
 35 described herein, including but not limited to modified Nef proteins which comprise a
 deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BglII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)ClaI (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the PacI site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with Bgl II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bg*III site. The clones were checked for the correct orientation of the gene by using

5 restriction enzymes *Dra*III/*Not*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its

10 isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)*Cla*I. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA

15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate co-

25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant

30 adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *PacI* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)ClaI pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*II releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the MRKpdeIE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*II site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*I. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)ClaI. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *Pac*1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at $\leq -60^{\circ}\text{C}$. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHPA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

EXAMPLE 22

5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-
25 bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca* I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac* I and *Bst* Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial
30 homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁷ vp and 10⁹ vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFN γ ELISpot analyses, respectively. For all rodent immunizations, the Ad5 vectors were
 5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadriceps muscles in 50 μ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following
 10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were
 15 collected from all the animals for RT ELISA and IFN γ ELISpot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁹ vp and 10¹¹ vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either
 20 10⁹ vp and 10¹¹ vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0)
 25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μ L of 1 μ g/mL HIV-1 RT protein
 30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 μ L of 1 μ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200 μ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was
 35 performed followed by 4-fold serial dilution. 100- μ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H_2SO_4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELISpot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF γ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5×10^6 /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μ L/well of either 5 μ g/mL purified rat anti-mouse IFN- γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 μ g/mL mouse anti-human IFN- γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μ L of cell samples ($4-5 \times 10^5$ cells per well) and 50 μ L of the antigen solution were added. To the control well, 50 μ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 μ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT ELISA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁷ vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers ^a			SFC/10 ⁶ cells ^b		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10 ⁷ vp	2	310419	301785	153020	1(1)	75(4)	2313(57)
			1	919	372	265	1(1)	72(9)	533(41)
2	MRKAd5hCMVFLpol (E3+)	10 ⁹ vp	2	1638400 ^b	0	0	2(2)	114(9)	2063(182)
			1	713155	528520	303555	1(1)	48(7)	733(89)
3	MRKAd5hCMVFLpol (E3-)	10 ⁷ vp	2	310419	386218	172097	0(0)	223(7)	2607(27)
			1	6400	14013	4393	10(8)	141(21)	409(28)
4	MRKAd5hCMVFLpol (E3-)	10 ⁹ vp	2	1638400 ^b	0	0	1(1)	160(13)	2385(11)
			1	1241675 ^b	396725	300661	0(0)	39(13)	833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNear or at the upper limit of the serial dilution; hence, could be greater than this value^cNo. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers ^a			SFC/10 ⁶ cells ^b		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10 ⁷ vp	2	174	70	50	1(1)	23(1)	1(1)
			1	132	42	32	0(0)	0(0)	0(0)
2	MRKAd5hCMVFLnef (E3+)	10 ⁹ vp	2	174	70	50	0(0)	61(7)	4(2)
			1	132	42	32	1(1)	62(7)	3(1)
3	MRKAd5mCMVFLnef (E3+)	10 ⁷ vp	2	132	42	32	3(1)	15(5)	5(2)
			1	115	46	33	3(2)	3(2)	4(2)
4	MRKAd5mCMVFLnef (E3+)	10 ⁹ vp	2	132	42	32	4(2)	83(13)	5(1)
			1	132	42	32	2(1)	29(2)	4(0)
5	MRKAd5mCMVtpanef(E3+)	10 ⁷ vp	2	132	42	32	3(2)	14(2)	5(1)
			1	100	0	0	3(1)	13(4)	10(3)
6	MRKAd5mCMVtpanef(E3+)	10 ⁹ vp	2	230	170	98	3(2)	145(29)	4(0)
			1	115	46	33	7(1)	151(14)	10(0)
7	Naïve	none	none	152	78	52	21(2)	18(6)	26(3)

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNo. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monkey #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IAPol(E3+) 10^{11} vp	99C100	1	0	0	1	38	31	0	52	146	0	49	715
	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IAPol(E3+) 10^9 vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	192	4	38	156	5	38	106
	99C201	8	5	21	6	62	62	0	18	32	1	14	65
MRKAd5hCMV-IAPol(E3-) 10^{11} vp	99D239	5	2	2	20	82	172	1	66	114	9	21	40
	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	236	1	24	264
MRKAd5hCMV-IAPol(E3-) 10^9 vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Naïve	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined
Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/mL				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IAPol(E3+), 10^{11} vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IAPol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IAPol(E3-), 10^{11} vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IAPol(E3-), 10^9 vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 11 vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 9 vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 11 vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 9 vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 ⁶ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 ⁶ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of
- 20 MRKAd5gag. PER.C6[®] cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were
- 25 harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

- Comparison of hCMV- and mCMV-FL-nef* - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6[®] cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 ⁶ cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10 ⁶ vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

20

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

- Materials and Methods* - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6[®] cells at a concentration of 0.2x10⁶ cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10⁶ cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

10

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

15

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 ¹³ vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 ¹¹ IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10^7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10^7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, $CD4^+$ -biased or $CD8^+$ -biased, and (b) boosting with the MRKAd5gag
30 construct produced in all cases a strongly $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific $CD8^+$ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag

Grp#	Priming T=0, 4, 8 wks DNA/5 mgs PBS (D101)	Boost T=28 wks MRKAd5gag(E3+) 10 ⁷ vp	Monk#	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
				Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1			CB5H CB6X AW3G	NA 0 5	NA 0 11	3 0 0	35 15 38	15 0 3	71 45 51	4 0 3	224 58 48	8 0 2	115 78 89	5 0 8	85 35 65	19 3 10	950 1705 989	0 1 0	316 755 395
2			CC1C CC1K AW3P CB5F AK8B	0 4 9 NA 9	4 0 8 NA 12	1 1 1 0 4	60 101 10 31 38	0 0 4 0 1	111 284 71 288 119	5 0 4 0 0	270 791 154 530 439	4 5 8 19 0	280 452 104 374 425	8 0 5 9 0	232 321 85 251 310	3 0 11 8 4	959 1915 838 1549 1229	18 1 6 20 5	1345 1099 241 1734 1354
3			AW20 CA4R CB58 CB5W CB7D	10 1 8 4 1	4 0 6 3 0	1 3 0 0 0	59 121 6 28 136	5 1 3 1 0	284 135 119 91 316	19 1 0 0 1	425 270 274 139 609	6 5 8 0 5	105 130 282 164 625	9 1 1 1 1	205 105 208 82 759	18 14 0 5 0	565 1384 838 543 2278	8 10 1 1 4	404 978 828 349 1831
4			88D201	3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0

NA, not available

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused
5 directly to the open reading frame of the IA pol gene (consisting of RT, RNaseH and
integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not
include the protease gene and the frameshift sequence, it encodes a single polypeptide
of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID
NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last
non-stop codon was ligated via PCR to a fragment that extends from the start codon
of the IAPol to a unique BamHI site. This fragment was digested with BstEII and
BamHI. Construction of gag-IAPol fusion was achieved via three-fragment ligation
involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII
fragment of the V1R-gagpol containing the entire ORF of gag-IAPol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

20 Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral
particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag;
(2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of
MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and
4.

30 The T cell responses against each of the HIV-1 antigens were assayed by IFN-
gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein
sequence of each antigen. The results (Table 25) are expressed as the number of spot-
forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that
respond to each of the peptide pools.

35 Results indicate the following observations: (1) each of the single gene
constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels
of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can
be mixed as a multi-cocktail formulation capable of eliciting very broad T cell
responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

5 **Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10 ¹⁰ vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10 ⁸ vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10 ¹⁰ vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10 ⁸ vp	AW38	1	-	19	30	-
		CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10 ¹⁰ vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10 ⁸ vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ¹⁰ vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ⁸ vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10 ¹⁰ vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10 ⁸ vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10⁶ PBMC.

WHAT IS CLAIMED IS

1. A recombinant adenoviral vaccine vector at least partially deleted in
5 E1 and devoid of E1 activity, comprising:
- a) an adenovirus *cis*-acting packaging region corresponding to from
about base pair 1 to between from about base pair 400 to about
base pair 458 of a wildtype adenovirus genome; and
 - b) a gene encoding an HIV protein or immunologically relevant
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region
corresponding to from about base pair 1 to about base pair 450 of a wildtype
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides
15 corresponding to between from about base pair 3511 to about 3524 to about base pair
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).
18. A cell comprising the adenoviral vector of claim 1.
19. Recombinant, replication-defective adenovirus particles harvested
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.
20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
21. An HIV vaccine composition of claim 20 which comprises a
10 physiologically acceptable carrier.
22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,
15 replication-defective adenovirus.
23. A method according to claim 22 wherein the cell is a PER.C6[®] cell.
24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
20 claim 21.
25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell
15 line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6[®] cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

49. An adenoviral vector in accordance with claim 9 wherein the gene
20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- 5 a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
 - 10 iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6[®] cell.

62. A method of generating a cellular-mediated immune response
15 against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs
15 corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising
i) a nucleotide sequence selected the group consisting of
SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and
20 SEQ ID NO: 15;
ii) a heterologous promoter operatively linked to i); and
iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10 75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

20

80. A method according to claim 79 wherein the cell is a PER.C6[®] cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

86. A multivalent adenovirus vaccine composition comprising
15 recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- 5
- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
 - c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
 - d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
 - e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
 - 10 f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
 - g) gag and pol, expressed independently from two individual vectors;
 - h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
 - 15 i) pol and nef, expressed independently from two individual vectors;
 - j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
 - 20 k) nef and gag, expressed independently from two individual vectors;
 - l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
 - m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;

and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with
claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to a single promoter; and the encoding nucleic acid sequences
operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

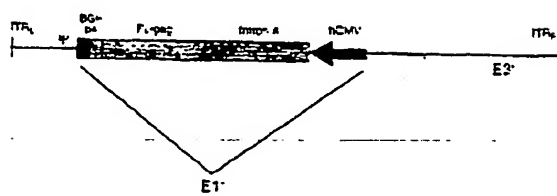


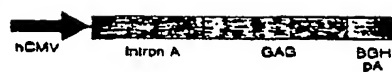
Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctgtagggctctgtgctgtctgtggtgagctggacaagtgaggagaagaatcaggctgaggccctgggtg
caagaagaagtacaaagcacaatgtgtgtggtccctccaggagctggagagggttctgtgaacctgtggc
ctgtctggagacctctgagggtgtagggcagatccttggtccagctccagccctccctgcaaacagggtctgagg
gagctgagggtccctgtacaacacagtggtctacctgtactgtgtgaccagaagattgatgtgaaggacaccaag
gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgtgtctggtg
acaggcaactccagccagggtgtccagaactaccccatgtgtgcagaacctccagggccagatgtgtgacccaag
gcatctccccccggaccctgaatgccctgggtgaagggtgtgtggaggagaaggccttctcccttgagggtatccc
catgttctctgcccctgtctgagggtgccacccccaggacctgaacaccatgtctgaacacagtggggggcccac
aggctgccatgcagatgtctgaaggagaccatcaatgaggaggctgtgtgagtgaggacaggctgtcatctgtgc
acgtctggccccatgtccccggccagatgaggggagccccaggggctctgacatgtctggcaccacctccacct
ccaggagcagattggctggatgaccaacaaccccccatccctgtgggggaaatctacaagagggtggaatcat
ctggggccctgaacaagattgtgaggatgtactccccacctccatccctggacatcaggcaggggccccaaggag
cccttcagggaactatgtggacagggttctacaagacctgaggggtgagcaggccctccaggagggtgaagaact
ggatgacagagacctgtctgtggtgcagaatgccaaacctgactgcaaggacctctgaaggcccttggggccct
ctgcccacctggaggagatgatacacgcttgcagggggtgtgggggcccctgtgtcaaggccagggctgtgt
gtctgaggccatgtccagggtgaccaactccggcccaatcatgactgagcaggggccaacttcagggaaccagag
gaagcacgtggaagtgctcaactgtggcagggtggccacattgccaaagactgtgaggccccagggaaga
agggtctctggaagtggtgtgagcagggtggccaccagatgaaggactgcaatgagaggcaggccaacttctgt
ggcaaaaatctggccctcccaaggggcaggccctggcaacttctccagtcaggccctgagcccacagcccct
cccaggagctcttcagggttggggaggagaagaccacccccaggcagaagcaggagcccatgacaagg
agctgtacccccctggccctccctgagggtccctgtgtggcaacgaccttctccagtaaaaataaagcccgggca
gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

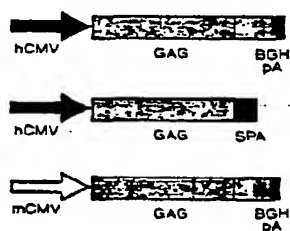


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

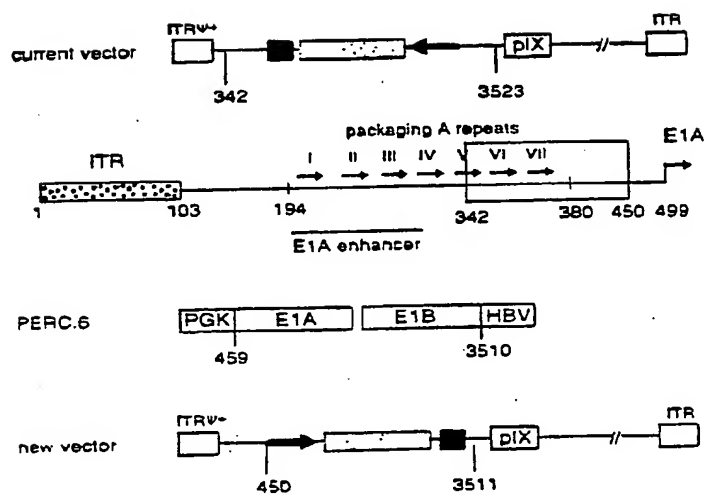


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

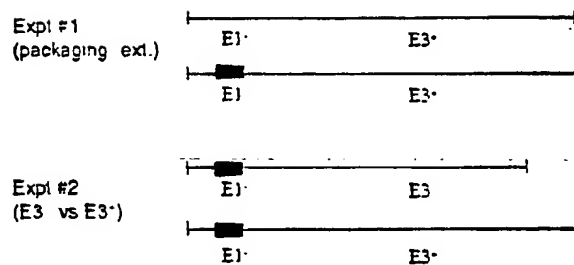


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

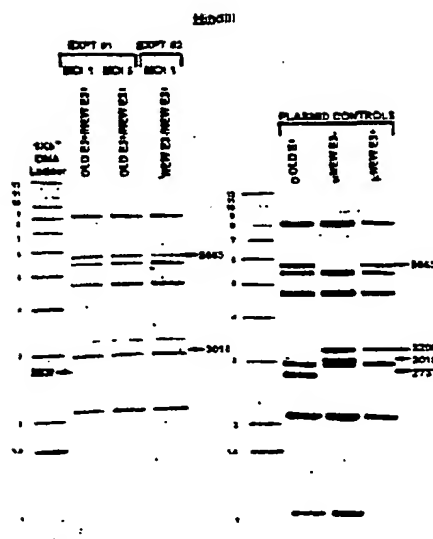


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

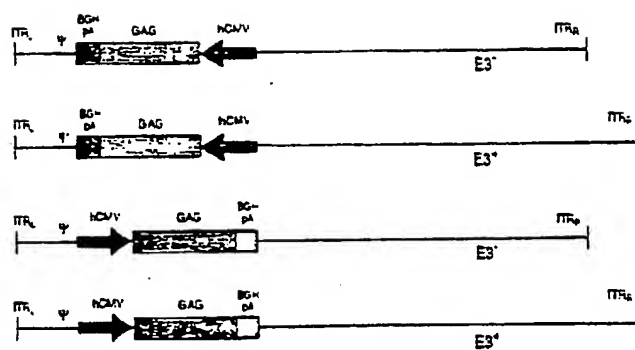


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3⁻ and E3⁺ backbones were constructed.

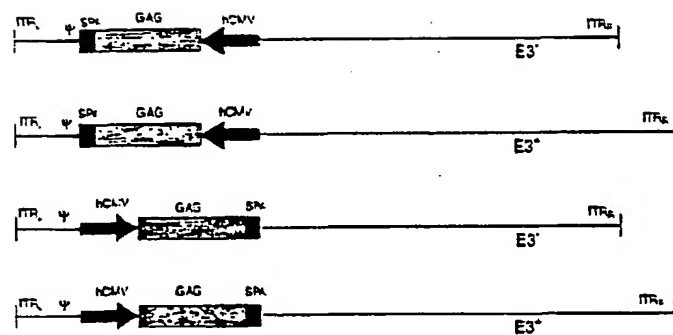


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

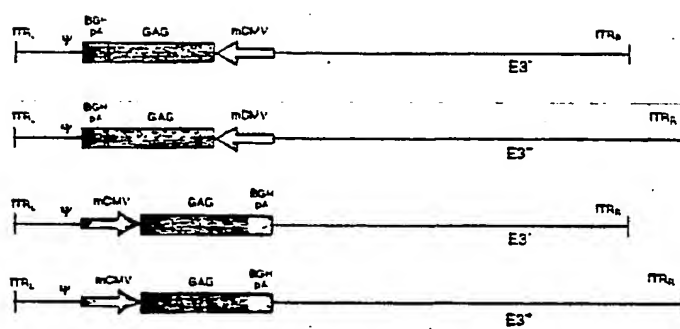


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3⁻ and E3⁺ backbones were constructed.

Plasmid mixing expt: (orientation)

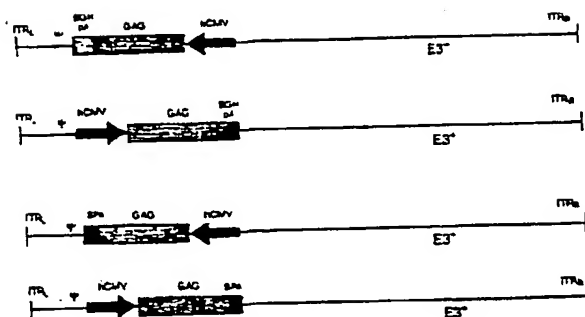


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

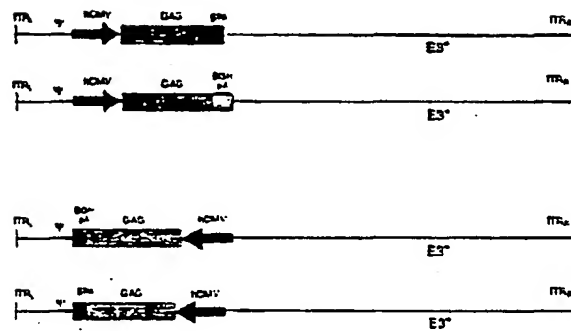


Figure 8B: Effect of polyadenylation signal

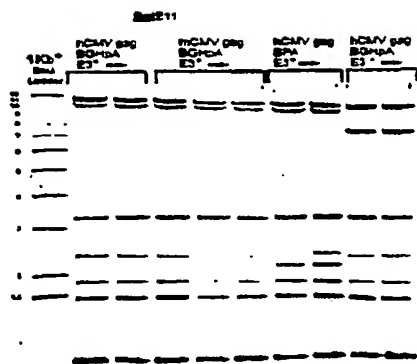


Figure 9: Viral DNA from the four Adgag candidates at P5, following *BstE11* digestion.

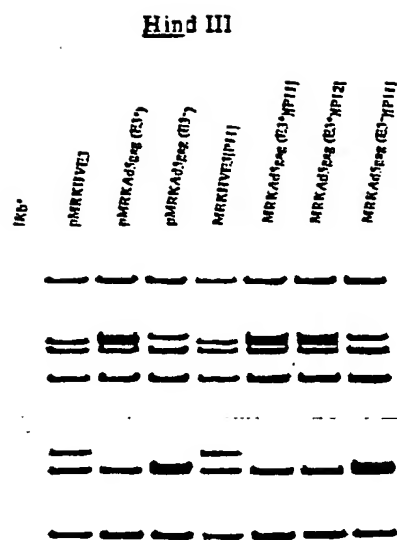


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

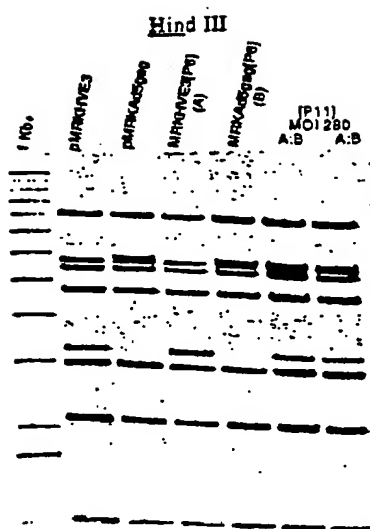


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

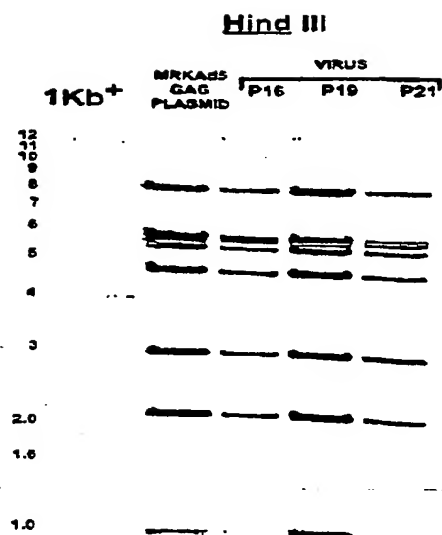
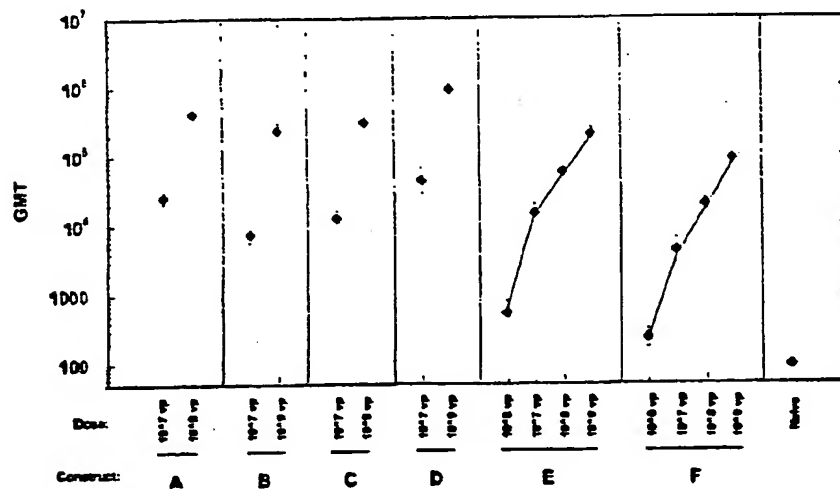


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

13
 Figure 13. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3⁻ bCMV-FLgag-bGHpA; (C) MRKAd5 E3⁻ bCMV-FLgag-SPA; (D) MRKAd5 E3⁺ mCMV-FLgag-bGHpA; (E) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



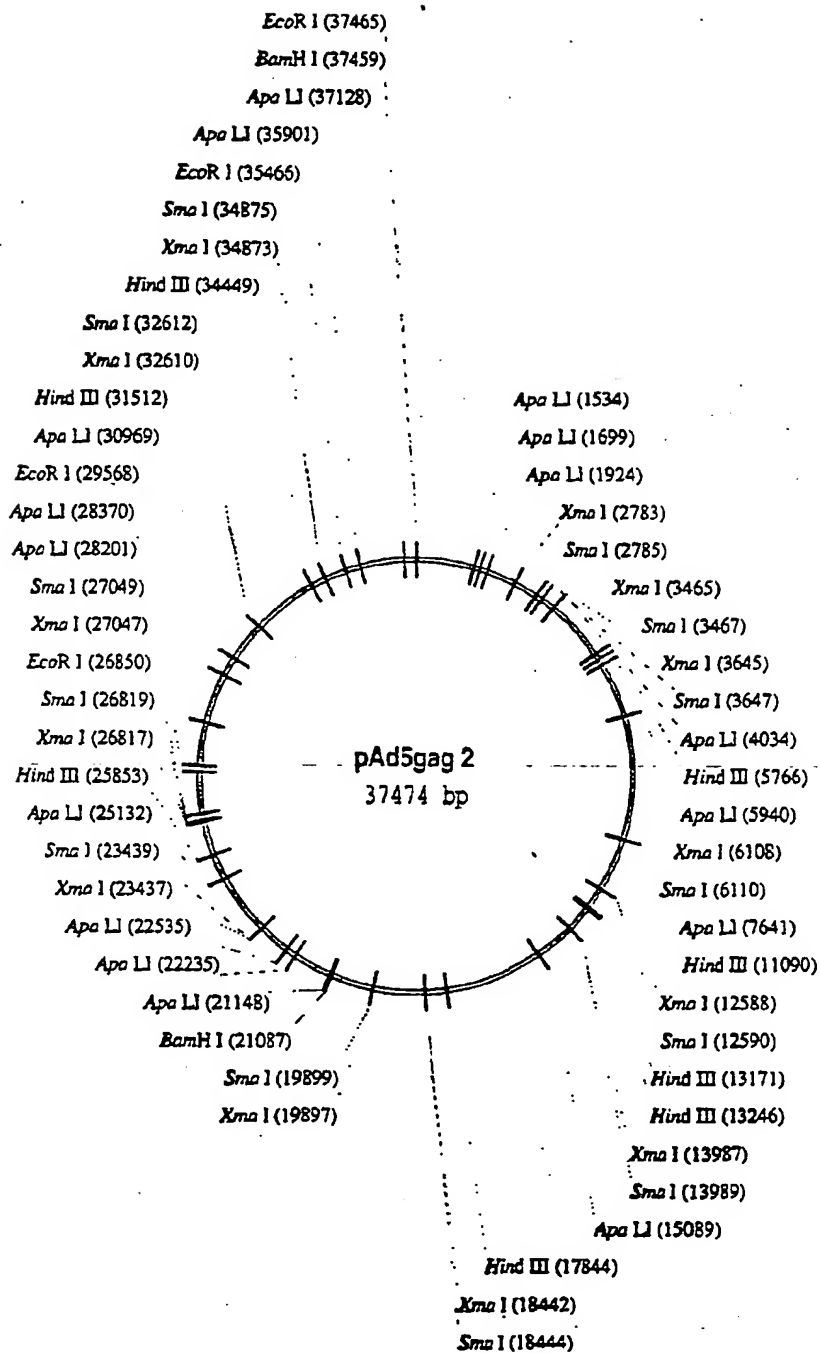


Figure 14

pMRKA150009 ME16082

1	TTCTTAATTA ACATCATCA	TAAATATACCT	TATTTTGTAT	TGAAATCAAT	ATCATATATTA	GGGCTGGG	TTTGTGACGT	GGCTGGGG	GTGGTAAGTG
	ANGAATTAAT TCTAGTAGT	ATTATATACA	ATAAACTTA	ACTTGTATTA	TACTATTACT	CTCCACCTC	AAACACTTCA	CCCGTCCCG	CACCTTCTT
101	GGCGGGTAC GTAGTAGTGT	GGCGTACCTG	TGATTTTCA	AGTTTATGAG	AGCAATATTA	AGCAATATTA	GTGGTANAG	TGAGCTTTT	GTATTTTCT
	CCCGCCACTG CATCATACA	CCCGTTTAC	ACTACATGCT	TACACCCCG	TTGTGTACAT	TGCTGTCTA	CACGTTTTT	ACTGCAAAA	CCACACCCG
201	GGTGTACACA GGAATGACA	ATTTTGTGT	GGTTTATGCT	GGATTTTCTA	GTAAATTTG	GGTAACTGCA	GTAAATTTT	GGCATTTTCTG	GTGGTANAG
	CCCATGTGT CTTTCACTGT	TAAAGCTG	CCAAATCTG	CCATATACAT	CAATTTAAAC	CGATTTGCT	CGATTTTAC	GGCTTTTCT	GGCTTTTCT
301	GAATTAAGAG AGTGTAAATC	ACTTATTAAT	ACACATATG	ATATTTTCT	ATATTTGCT	AGTGGCTG	GGCTTTTCT	GGCTTTTCT	GGCTTTTCT
	CTTATTTCTT TTTTCTTTAG	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT
401	CAGTGTGTT TCTCAGTGT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT	TTTCTTTT
	GTCCACAAA AGATCCACA	AAATCCGCA	GGCCAGTTT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT
501	ATATGTACAT TTATTTGCT	TCATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT	CAATTTTCT
	TATACATGTA ATATTAACG	AGTACGCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT
601	TAGCCATAT ATGAGTTTCT	GGTTTACATA	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT
	ATCGGTATTA TACCTCAAG	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT
701	GTTCCTATTA TACCTCAAG	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT	CCATTTTCT
	CAAGGTATC ATTGCTGTTA	TCCCTGAAAG	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT
801	CAAGTACCTC CCTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT
	GTTCATGCTG GGTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT	GTATTTTCT
901	GGTATTTTCT ATGCTATTTA	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	GGTATTTTCT TACCTCAAG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1001	ATTTAGCTCA ATGAGTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	TAACTGCAAT TACCTCAAG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1101	TACCTGCTCA GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	ATGCAACCTT CCGATATAT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1201	CCGATCCAGC CTGCGGGC	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	GGTATTTTCT GAGCGGGC	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1301	TGCTGAGCTG GACATGCTG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	ACCACTGAGC CTGTTTCA	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1401	TTTCTGCTGA ACCCTGCTG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	AAATGACACT TGGGACCTG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1501	CCCTGTACAA CACATGCTG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	GGGACATGTT GTGTCACCA	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
1601	GTCCAAAGAG AAGCCGAGC	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT
	CAGGTTCTTC TTGCGGCTG	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT	GGTATTTTCT

Figure 15A

pMRK41549 MER62

1701 CACCAGGCCA TCTCCGCCCG GACCCCTAAT CCCATCATCA AATCATATCA GAGAAAGGCC TTCTCCCTCG AGGTATATCC CATTTCTCT GGCCTCTCTG
 GTGTCTCGGT AGAGGGGGGC CTGGGACTTA GGAAGCCACT TTAAAGACT CTCTTCCCG AGAGGGGC TCCACTAGCG GTACAGAGA CGTACAGAC
 1801 AAGGTGCGAC CCCCAAGGAC CTGAAACTA TATTAAGAC AATTAAGAC CATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 TCCACAGGCG GGGGAGGCG GACTTTATG AATTAAGAC AATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 1901 TGAAGGAGAC AGGTGAGAC CTATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 ACTCACCTCG TCCAGGAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2001 CAGGAGCAGA TTGGCTGAT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2101 ACTCCCCCAG CTCCTATCTG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 TGAAGGAGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2201 CCAGGAGGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2301 GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2401 TGAAGGAGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 ACTCACCTCG TCCAGGAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2501 GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2601 GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 TGAAGGAGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2701 AATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 TGAAGGAGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2801 GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 2901 TGAAGGAGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 AATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 3001 GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 CCTACGCCAC CGGATATACC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 3101 TGAAGGAGCG GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 AATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 3201 GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC
 GTCTCTCTCT AACCGACTA CTGGTCTCT GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC GATTAAGAC

Figure 15B

[illegible]

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[illegible]

Figure 15D

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6501 GCGTACACCA CGAAGAGGC GTACAGATCG CCGACCTTGT TACATAGCTC GCGCGTAGC TGCACGTCTA GGGCCACCTA GTCCAGTGTG TCTTTGATCA
 CCGAGTGCCT GCTTCTCTCG CATCTCTAGC GGTCTCCAGC ACTGTTGAG GCTCTACTCG AGCTGTAGAT CCGCGTCAT CAGGTCCCAA AGGACTACT
 6601 TGTCTACTTT ATCTCTGCCC TTTTCTTCC AATCTCTGAG GTTACAGTCA ATCTCTGAG CAGTCTTCTA GTACTCTTGG ATCCGAAACC CCGCGGCTT
 ACNOTATGAA TAGGACAGCG AAAAAAAGT TCTCTAGCTC GATCTCTGAG TGTACAGGCG CCAGAAAGCT CAGCGAAACC TAGCGTTTGG GACCGCGG
 6701 CGAACGTAA GAGCTAGCA TGTATAGCTG GTTAGAGGCG TGTATAGGCG AATCTCTGAG TGTACTGCTT AGCGGTAGG CCTGCGCGCG CTTCGCGGAG
 GCTTCCCTTT CTGCGATCTT ACATCTTAC CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG
 6801 GTAGGTGAGG TAGGCGCAAA GGTGTCTCTG ACTATGCTT TGTACTGAA ATCTCTGAG TGTACTGAG TGTACTGAG TGTACTGAG TGTACTGAG
 GCTGACACCG ACTGCGCTTT GGTGAGGAG TGTACTGAA ATCTCTGAG TGTACTGAG TGTACTGAG TGTACTGAG TGTACTGAG TGTACTGAG
 6901 CCGTCTGCTT TTTGAGGAG GGTATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 GCACCGGAA AACCTTGGC CTAAACCGT CCGCTTCCA CTTTACGAC TTTTACGAC TTTTACGAC TTTTACGAC TTTTACGAC TTTTACGAC
 7001 TCCGCGACC TCGGAGCGT TGTATGAGG TGTATGAGG TGTATGAGG TGTATGAGG TGTATGAGG TGTATGAGG TGTATGAGG TGTATGAGG
 AGCGCGCTGG ACGCTTGGCA ACATTTAAG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG CACTGCGCG
 7101 GGTATGCGCT TGTATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 CCGTACGGA ACTACCTTCC GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 7201 GGTATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 CCAAGCTTCC GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 7301 GGTATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 CCACTACGTC ATCTTCCAT CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG
 7401 AACTTACGTA CCGAGAGTA GCGAGAGTA GCGAGAGTA GCGAGAGTA GCGAGAGTA GCGAGAGTA GCGAGAGTA GCGAGAGTA GCGAGAGTA
 TGTATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 7501 GATGAGGAG GATGAGGAG AACTGAGT CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG CCGCGAGAG
 CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG CTAGCTGCG
 7601 GTCTGCGCT TGTATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 CAGGAGCGA AACTTCTT CAGGAGT CAGGAGT CAGGAGT CAGGAGT CAGGAGT CAGGAGT CAGGAGT CAGGAGT CAGGAGT
 7701 GCGAATTTGA GCGCTGCG TCGGCTT GCGCTT GCGCTT GCGCTT GCGCTT GCGCTT GCGCTT GCGCTT GCGCTT
 CCGTTAACT CCGGAGCG CCGGAGCG CCGGAGCG CCGGAGCG CCGGAGCG CCGGAGCG CCGGAGCG CCGGAGCG CCGGAGCG
 7801 GAGAGCGAG GCGCGCGAG CCGAAGTCC AGATGCTCC GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG GATATGAGG
 CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT CCGGCTGCT
 7901 CCGCGCGAG GATGAGT CCGGAGT CCGGAGT CCGGAGT CCGGAGT CCGGAGT CCGGAGT CCGGAGT CCGGAGT
 GAGGCGCG CAGTCCAGT CCGGCTGAG CCGGCTGAG CCGGCTGAG CCGGCTGAG CCGGCTGAG CCGGCTGAG CCGGCTGAG CCGGCTGAG
 8001 TGTATGAGG CCGGCTGAT GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG
 ACCAGCCAG CCGGAGCTA CCGAGCTT CCGGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG GCGCTGAG

Figure 15E

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9701	ACAAAGCCGT GGTATGCGCC GGTGTGATG GTTAAATTC AGTTGCGAT ACTGACGAC TTAAAGGTCT GGTGACCGG CTGCGAGAGC TCGTGTACT	XbaI
9801	TTGTTGCGCA CCATAGCGG GCACACTAC CCAATTCAG TCAACGGTA TTTCCTGTC AAATGCGAGA CCACTGCGC GACGCTCTG AGCCACATG	XbaI
9901	TCAGACGCGA GTAAAGCCCT GAGTCAAAAT CTTATCTCT GTAACTCTC ACTAGTACT GTATGCGC CAAAGATGC GGCATCGGCT GGCCTTAGAC	XbaI
10001	ACTCTGCGCT CATTCGGGCG CTCAGTTTAT GCATCACTAA CATTACAGTC TATCTCATTA CCATAGCGG GTTTTTCAGC CCGCGCGCGA CCGCCATCTC	XbaI
10101	GGCCGAGCGT ACGTGTGCGG GGCCTCTGAG GTCAGACTCT TGTAAATTA TCGGACTATA TCGTATGATG TACCTGACA TCCAGTGTAT GCGCGGCGAG	XbaI
10201	CCCGGTGCGA TCCGACCGGC CCGGAGGCGC CCGCTCTAGA AGTTGTATTT CCGCTACTAT ATGCACTCTAC ATGGACTCTT AGGTCCTACTA CCGCGCGCGC	XbaI
10301	GTGCTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG	XbaI
10401	CACCACTCC CCGCGCGCTT CAGCGCGCTC GGCAGGCTCT ACAGCGCTCT GGCAGGCTCT ACAGCGCTCT GGCAGGCTCT ACAGCGCTCT GGCAGGCTCT ACAGCGCTCT	XbaI
10501	AAATGTTGAC GCTCTAGACC GTGCAAAAGG AGAGCTCTTA AGCGCTCTT CTTGCTGCTT CTTGCTGCTT CTTGCTGCTT CTTGCTGCTT CTTGCTGCTT	XbaI
10601	TTAGCAACTG CCGATGCTCG CACGTTTCTC TCTCGGACAT TCGCGCTTA GAGGCTCTTA GAGGCTCTTA GAGGCTCTTA GAGGCTCTTA GAGGCTCTTA	XbaI
10701	GGGTTGAGC CCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG	XbaI
10801	CCCAAGCTCG GGCATAGGC CCGCAGGCGG CACTAGTAC GGTATGCTG GGTATGCTG GGTATGCTG GGTATGCTG GGTATGCTG GGTATGCTG	XbaI
10901	TTTCAGGCGC GCGGCTGCTT GCGCTAGCTA GCGCTAGCTA GCGCTAGCTA GCGCTAGCTA GCGCTAGCTA GCGCTAGCTA GCGCTAGCTA GCGCTAGCTA	XbaI
11001	AAACCGAAGG AAGTGTGCGG GTGAGCGCGA ACATGCGCT CCGCTGCGCT CCGCTGCGCT CCGCTGCGCT CCGCTGCGCT CCGCTGCGCT CCGCTGCGCT	XbaI
11101	GTGCTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG CCGTGTGAGG	XbaI
11201	CTCATGCGGC AGCTGTTCTT TATAGTCGAG CACAGTATAT ACAGTATAT ACAGTATAT ACAGTATAT ACAGTATAT ACAGTATAT ACAGTATAT	XbaI

Figure 15g
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12901 GCAATGATATG CCTCAAAACG GCGCTTTATC AACCTGCTTA TTACTACTCT GATTGCTG GCGCTGCTA ACCCTGATTA TTTCACCAAT GCGATCTTGA
CCTTACATAC GAGTTTGGG CCGCAATATG TTGCTGATTT ACTTATGCA CTTATGCTT GCGCTGCTA GCGCTGCTA TTTCACCAAT GCGATCTTGA
13001 ACCCGCATG GCTACCGCGC CCGCTTTCT ACACCTGCTT ATTCGCTGCTT CTTATGCTT ACTATGCTT GCGCTGCTA GCGCTGCTA TTTCACCAAT GCGATCTTGA
TGGGCTGAC GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG GATGCTGCG

13101 TTCTCTCTCA CCGCAGACCT TGTCTAGCTT GTCACAGCT GAGCAGGCT GAGCAGGCT GAGCAGGCT GAGCAGGCT GAGCAGGCT GAGCAGGCT GAGCAGGCT
AAGCGCTT GCGCTGCTG ACGATCTCA CTTGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT

13201 CTAGCGCTG CCGCGCGCG GTCAGATCT ACTAGCTT TCCAGCTT TCCAGCTT TCCAGCTT TCCAGCTT TCCAGCTT TCCAGCTT TCCAGCTT TCCAGCTT
GATCGCGGAC GCGCGCGCG CAGTCTAGCA TCACTGCTG AAGCTTCA CTTATGCTT GCGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT GCGCTGCT

13301 AAGAGGATGA CCTAAACAC TCGCTGCTG ACGCTGCTG CCGCAAAAC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG
TCTCTCTCAT GATTTTGTG ACGAGGAGC TCGCTGCTG GCTTTTGTG GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

13401 GATGATATG AAGATATG CCGAGGCA CAGGAGGCT CCGCTGCTG GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
CTCATCTTACC TTCGCTGCT GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG

13501 GAGAGGATG ACTGCGGAGA CCGAGGAGC GTCCTGCTT TCGAGCTT TCGAGCTT TCGAGCTT TCGAGCTT TCGAGCTT TCGAGCTT TCGAGCTT TCGAGCTT
CTCTCTCTAC TAGCGCTCT GCTGCTGCT CAGAGCTTAA ACCCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG

13601 AAAAAAATA GATGATGCA AATAAATA CTGACCAAG CCGCTGCTG GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
TTTTTTTTT GATGATGCT TTTTTTTT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

13701 TAGGAGGCT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT
ACTCTTCTCA GAGAGGGA GATGCTGCT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT CCGCTGCTT

13801 GTCTCTCTG GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
CAGGAGGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

13901 AAGATGCTG GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
TCTGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

14001 CACAGGCTG ATGATGCTG ACCGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
GTGCTGCTG GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

14101 AATAGTTTA AGCGCGGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
TTATTCATAT TCGCGGCT CAGGAGGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

14201 ACTGCTGCTA GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
TATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

14301 GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
CCTGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

14401 AATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT
TATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT GATGCTGCT

Figure 15I

pmrkad5gag MER682

14501 CCTACGATGA TCTGAGGCT GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 14601 GGTACTACT AGACCTGCA CATTCTGAG GCGCTGACA CTACATCTG CAGATCTGTC GTCTGACACTT TCTACTGTCG CTCTGTCGCG CCGCACGCGT
 14701 AGCGGCAAC MACAGAGTG GCAAGCGCG GTACAGAGC TCAAGTCTG CAGATCTGTC GTCTGACACTT TCTACTGTCG CTCTGTCGCG CCGCACGCGT
 14801 TCGCGCTCG TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 14901 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15001 TCGCGCTCG TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15101 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15201 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15301 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15401 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15501 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15601 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15701 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15801 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 15901 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC
 16001 CCGTCTGTC TTCTGTCAC CCGTCTGTC GTTACATTC CCGACTCTT CATTCTGAC GTTACAGAG GTCTGACACTT AGATGACAGC GATACAGGCG GCGGTCTGTC

Figure 15J

pMRKd5gaq MER6B2

16101	CCAGGTCATC GCGCCGGAGA TCTATGCGCC CCGAAGAAAG GAAAGACAGG ATTACAAACCTA AGCGGGGTCA AAGAGAGAAA GAAAGATCAT GATCCAGTAG CCGCGGCTCT AGATACCGCG GGGCTCTCTC CTTTCTCTTC TAAATCTCTG GACTTTGAT TTGCGCCAGT TTCTCTTTT CTTCTACT	SalI
16201	GATGATGAG TTGACGACGA GGTTCACACT CTCACACCTA CTCTCTCCAG GCGATGCTTA CAGTGGAAAG GTCCAGGCGT AAGAGTGT TTGGGACCC CTACTACTTG AACTGCTGCT CCACCTTGAC GACGTGGGAT GAGCTGCTTA GTTACCTTTC CAGCTGGCCA TTTTTCACAA AACCTGTG	SalI
16301	GCACCAACCT AGTCTTTAGC CCGCTTAGC CCGTACACCG CACTTACAA GCGTCTATAG CCGTCTATAG ATGAGGTCTA CCGCGACCTAG GACTTGTCTG AGCAGGCGCA CGTGTGCGCA TCGAAGATTC GCGCACTCG CCGAGTGGCG CTGATGTTTC GTTCACATAC TACTTCCACAT GCGCTGCTC CTGACGACAC TCGTCTCGTT	SalI
16401	CGAGGCTCT GAGGAGTTTG CTTACGGAAA GCGGCATAG GACATCTCTT CTTTCTCTCT GCGAGGAGCG AACCCAGCAC CTAGCCTAAA GCGCGTAACA GCTCCGCGAG CCGCTCAAC GATGCGCTTT CCGCTATTTC CTGTAGGACC GCAACGCGCA CCTGCTCCCG TTGGGTTCTG GATCGAATTT CCGGCAATTT	KpnI
16501	CTGACGAGG TGTGTCGCGC GCTTCACCG TCCGAGAAA AGCGCGCT AAGTGTCCAG TCTGTGACT TGGCACCCAC GGTGAGGCTG ATCTATCCCA GAGTCTGTC ACAGCGCGCG CGAAGCTGCG AGCTTCTTT TCGCGCGGTA TTTCGCTCTA AGACNCTGA ACCGTGCGTG GCACGTGCAC TACCTATCTG	KpnI
16601	AGCGGCAAGC ACTTGGAAT GTCTTGTAAA AATGACCGT GAACTCTAT GCGACCGCG AGGTCTGCTT CCGCGCAATC AGCGAGGTG CCGCGGACT TCCGGTCCG TCACTTCTTA CAGAACCTTT TTACTTGCA CTTTGGACTC GACCTGGGCG TCCAGTGGCG CCGCGGTTAG GCGCGTCC CCGCGGCTGA	KpnI
16701	GCGGTTGCG AGCTGACCTG TTCAGATACC CACTTACCAT AGCACTATTA TTGCCACCG CACAGAGGCG ATGAGACAC AACGTCCCG GGTTCGCTCA CCGCACTGTC TGGCACTGCG AAGTCTATCG GTGATGCTCA TCGTGTCTAT ACGGTGCG GTGTCTCTCG TACCTCTCTG TTTCAGGCG CCAAGCGAGT	KpnI
16801	GCGGTGCGCG ATCGCGCGCA TACGCGGCA CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT GCGCACTGTC TCGCACTGTC CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT	KpnI
16901	GCGGTGCGCG ATCGCGCGCA TACGCGGCA CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT GCGCACTGTC TCGCACTGTC CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT	KpnI
17001	CACCTACCG CCGCAAGAG CAGCACTAC CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT GCGCACTGTC TCGCACTGTC CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT	KpnI
17101	GCGGTGCGCG ATCGCGCGCA TACGCGGCA CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT GCGCACTGTC TCGCACTGTC CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT CAGCTGCT	KpnI
17201	ATATGCGCT CACCTACCG CCGCAAGAG CAGCACTAC CCGGTTGAGG AGATACGCG CCGCTGCGCG GCTTACAGAG CTCACAACTG GATCTGCTT TATACCGCGA GTGACCGCG GAGCAAGG GCGCACCGCG TAAAGCTCTT TCTTACGCTG CATCTCTCTC GTACCGGCGG GTGCGGCGCT GCGCGCGCTA	SphI
17301	CGGTGCGCG CACCAACCG CCGCGCGCG GTCGACCGT CTATATCTG CCGGTATCTT GCGCTCTCTT ATTCACCTGA TCGCGCGCG GATTCGCGCT CGGAGACCG GTGCTGCGCG CCGCGCGCG CAGCGTGCA GCGTACGCG CCGCATAGCA CCGGAGGAA TAAAGTACT AGCGCGCGG CTACCGCGG	SphI
17401	GTGCGCGGAA TTGCTACCTT GCGCTGCG CCGCGAGAG ACTATTTTAA ACGATCTTC ATGTGTGAAA TATGTGTGAA AAGGTCTGGA CTCTACGCT CACCGGCTT ACGTATGCGA CCGGACGTC CCGGTCTCTG TGACTAATTT TTGCTTCAAG TACACCTTTT TAGTTTTTAT TTTCAGACCT GAGAGTCTGCA	EcoRV
17501	CGCTTGTGCT TGTATCTATT TTGTAGATG GAGACATCA ACTTTGCTTC TCTGTCTGCT GCGACGCTT CCGCGCGCTT CATGCGAAC TCGCAGATTA CGGAGCGAG ACATTGATAA ACGATCTTAC CTCTGTGAGT TGAGACGAG AGACCTGCTC GCTGTGCGA GCGCGCGGAA GTACCGCTTGT ACCGTCTAT	EcoRV

Figure 15K

[illegible]

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19301	AGMAGTANB	GGCCNACAT	CTATCTCCMA	CAGGCGTAAAT	TACATPCTGCTT	TATGGRANAA	TTTTATPCTGT	CTANPCTATP	ACACAGACNE	GGGTAAATATP
19401	TCPTGATATAC	CGGTGTGTTA	GATACGGGCTT	GTCTGTATTTA	ATPACAGTANA	ATCTCTCTTT	ANANATACCA	GATTACATMA	TGTTCTGCTG	CCCATATATAC
	GGTGTCTCTG	GGGCGCANZ	ATCGTAGTTC	AAATCTTTTG	TAGATTCTGA	AGACAGNAAC	ACAGAGCTTT	CATACAGCTT	TTCTCTGTAT	TCCATATGATG
	CCACAGACC	GGCGGTCTG	TAGCGTCAAC	TTACAGACAC	ATCTAAATCTT	TCCTCTCTTG	TGCTCTGANA	GTATGCTGA	AAACGACATA	AGTAAACCA
19501	ATAGAACAG	GPACTTTTTT	ATACAGAAATC	AGCGCTATTTA	CAGCTATATAT	GGCATATPAA	CTATATPCTA	AAATCTATGA	ACTGAGACTA	AACTTCCAAA
	TATCTGCTT	CATGAAAGA	TACAGCTTAG	TCCAGAGACT	GTGCTATNTA	CTTATACATAT	CTTATATACCT	TTTATGATCT	TGACTCTTAC	TTGAAATTTT
	TTACTGCTTT	CGCTAGGZAG	GTCTATATTA	TACAGAGACT	CTTACAGACT	TAAAACTATA	ANACAGCTAG	GAATAATGAT	GGGAAANAGA	TGCTACAGAA
19601	AAATGADMA	CGCTAGGZCT	CACACTAAAT	ATGCTCTCTA	GAATCTCTCC	ATTTGZATP	TGCTCAATCT	CTTTTACCTA	CCCTTTTCTT	ACGATGCTCT
	TTTTACAGATA	AAATGTAAAT	AAAGATTGTA	AAATATTTTG	CCATGTAAAT	CAATCTAAAT	GCNACCTTT	GGAGAAATTT	CCCTCTACTCC	AACTATGCGT
	AAAGCTATP	TTTTACTTTA	TTCTCANCT	TTATATTAAC	GGTACCTTTA	GTATAGATTTA	CGTPTGACA	CTCTTTTAA	GGACATGAG	TGTATATGCT
19801	TGTTATTTGCT	CGACAGCTTA	AGTACAGTC	CTTCTGACGT	AAANATTTCT	GATATCTCTA	ACCATCTACT	CTCATGTAC	ACACAGATG	TGTATCTCTT
	ACATAAACG	CGCTGTTGAT	TTCTATGTCAG	GAAGGTGTCA	TTTTATMGA	CTATGCTGTT	TGCTAGATCT	GATGTACTTG	TTGCTCTAC	ACCGAGGCT
	GGTATGTGZAC	TCGTACATTA	ATGCTGTGTC	AGCTGTCTCC	CTTCTACTATA	TTGACAGACT	CAACCCATPT	AACCACTACC	GCATCTCTG	CCCTGCTGAC
19901	GAATACACTG	ACGATGTAAAT	TGCMACCTG	AGCTGACAG	GAATCTGTGA	CTTCTGTGTA	CAACTGTGTA	TTGCTGTG	CGTTACGACC	GGACCGGATP
	CGATCAACTG	TGCTGGGCA	TGCTGCTATP	GTGCGCTCTC	ACATCTCAAT	GGCTCAGNAG	TTCTTTTCCA	TTAAANACT	CCCTCTCTG	CGGGCTCAT
	GGGATTTACA	ACGACCCCTT	ACAGCGATA	CAGGGGAAG	TGTAAGTCCA	CGGAGTCTTC	ANANACCGT	AATTTTTGA	GGANAGGZAC	GGCTCGGATTA
20101	ACACTTACGA	GTGZAACTTC	AGCMGZATG	TTATACATGCT	TCCTTAGAGC	TCCCTAGGA	ATGACTCTAG	GGTGTACGA	GGCACCATTA	AGTTTGTATP
	TGTGZATGCT	CACCTTGTAG	TCCTTCTTAC	ATPTCTTACCA	AGAGTCTCTG	AGGGATCTCT	TACTGTGATTC	CCAACTGCTT	CGGTCTGTAT	TCANACTATP
	CATTPTGCTT	TAGCGTACCT	ATGCTCTCCAT	GGCCACACAC	ACGCTCTCCA	AGCGTTGAGC	CATGCTTAGA	AAGCACACCA	ACGACCCAGTC	CTTTTACGCT
20201	GTAAACGGA	ATCCGZTGA	AGAAAGGZTA	CGCGCTCTG	TGCTAGAGCT	GGCANCTCCG	GTACGAACT	TTGCTGTGCT	TGCTGTCTAG	GAANTTCTT
	GTATCTCTG	CGCCACACAT	GCCTCTACCT	ATACCGGCTA	AGCTTACCAA	CGTGGCCATA	TCATCTCTCT	CCCGCAACTG	GGCGCTTTC	GGCGCTCTG
	ATAGAGAGCC	GGCGTGTGTA	CGAGATGGA	TATGZGCTT	TGCGATGCTT	GCACGGGTAT	AGGTAGGGA	GGGCTTGTAC	CGCTCGAAG	GGCGGCTGZ
20401	CTTCTACGCG	CCTTAGACT	AGCTAACCC	CATCACTGCG	GTGCGGCTAC	GACCTTTATP	ACACCTACTC	TGCTCTTATA	CCCTACTTAG	ATGCAACTTT
	GGAGTGGCG	GGAAATCTGA	TTCTCTTGG	GTATGTACCC	GTGCGGCGATG	CGCCCTATTA	ACGCTATGAG	AGGAGATAT	GGATGTGAT	TACTCTGTA
	TTACTCTAC	CACACTCTTA	AGAAATGGC	GTATCTACTT	GACTCTCTCT	CTGGAATTA	TGCGAATCAC	CGCTCTCTTA	CCCCCAACGA	GTTTTAAATP
20601	AAATGZATG	TTGACGCGGA	GGGTATACCG	GGTGTACCCG	GTGTCTACTG	ACCTTTACTG	GGGAGCAAT	GGGGTGTCT	CAANCTTTAA	
	TTGCGZATG	AACTGCGGCT	CCCAATCTTG	CGATCTACCC	GTATATGANA	CTGAGNAGAC	AGBNACCATG	TTTATCCAGTC	ATTTGATATG	TTAACGATG
	AGGCTCTCTA	TATCTCCAGAG	AGCTACAGG	ACCGCATGTA	CTCTCTCTTT	AGAACTTCTC	AGCCCATGAG	CGCTGAGCTG	GTGZATGATA	TTATATACAA
20701	TCCGAGAT	ATAGGCTCT	TGGAATGTTCC	TAGGCTATAC	GAGTAAAGAA	TCTTTTGAGG	TGCGGTACTC	GGCATCTCAC	GATTTATATP	
	GGACTACCAA	CAGGTGGZCA	TCCCTACACCA	ACATACACAC	TCCTGTATTTG	TTGCTTACTT	TGCGGCTACC	CACTAGGCTTA	CACCTCTTAA	
	CCTGATGCTT	GTCCACCCCT	AGGATGTGCT	TGCTGTCTTG	AGACTTAAAC	AACTGATGGA	ACCGGCTG	TAGCGGCTTC	CTGTCTCGAT	GGGACGATTC
20901	TTGCGCTATC	CGCTTATAGG	CNAGACCTTA	GTTCACAGCA	TTATCCCAAGM	AAATTTCTTT	TCTCATCTGA	CCCTTTGCG	CATCTCCATTC	TCCATGTATP
	AAAGGZATAG	GGZAAATATCC	GTCTTGCGCT	CNACTCTCTG	AAATGZTCTT	TTTTCAAGMA	AGGCTAGCGT	GGZAAACCGC	GTAGGZTAAAG	AGCTCATATP

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21001	TTATGTCCAT	GGGCGACTC	ACAGACCTG	GCAMACCT	TCCTATGTC	ACTGCTCC	ACGGCTACA	CATGCTTT	GAGGTGATC	CCATGACGA
21101	AATACAGGTA	CCGCGGTAG	TCCTGACCC	CGATTGAGA	ACAGATGTA	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT
	GGCAGCCCT	CTTTATGTT	TTTATGAGT	CTTTAGCTG	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT
	CGGTGCGAA	GAATACAAA	ACAACTTCA	GAACCTGAC	CAGGACATG	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT
21201	TTGCGCGGCA	AGCCACAC	ATAAGAGC	AGCAACATC	ATCAACATC	ATCAACATC	ATCAACATC	ATCAACATC	ATCAACATC	ATCAACATC
	AGCGGCGCT	TTGCGGTG	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT	TTTATGCT
21301	TTGTTGTG	CCATATTTT	TTGCGACCTA	TTTACAGCC	TTTACAGCC	TTTACAGCC	TTTACAGCC	TTTACAGCC	TTTACAGCC	TTTACAGCC
	ACCAACACCC	GTATATAAA	ACCGTGGAT	ACTGTTGCG	AAAGTTGCG	AAAGTTGCG	AAAGTTGCG	AAAGTTGCG	AAAGTTGCG	AAAGTTGCG
21401	GAAGTGGG	GCATGCTAC	CTACCGGAA	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG
	CTCTGACCC	GCATGCTAC	CTACCGGAA	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG	CGGACCTTG
21501	AGGTATACCA	GTATGAGTAC	GAGTACCTC	TCGCGCTAG	TCGCGCTAG	TCGCGCTAG	TCGCGCTAG	TCGCGCTAG	TCGCGCTAG	TCGCGCTAG
	TCCAATATGT	CAAACTCAT	CTCAGTACG	ACCGGATC	ACCGGATC	ACCGGATC	ACCGGATC	ACCGGATC	ACCGGATC	ACCGGATC
21601	GGGCGCCAC	TCGCGCTC	GTGAGTAT	CTCTGCTAG	CTCTGCTAG	CTCTGCTAG	CTCTGCTAG	CTCTGCTAG	CTCTGCTAG	CTCTGCTAG
	CGCGGCTTG	ACCGGCTGA	CACCTGATA	GACGACGAC	AAAGGCTG	AAAGGCTG	AAAGGCTG	AAAGGCTG	AAAGGCTG	AAAGGCTG
21701	CTTATATACG	CTGATCCCA	CTGATCCCA	ACAGTCCCT	ACAGTCCCT	ACAGTCCCT	ACAGTCCCT	ACAGTCCCT	ACAGTCCCT	ACAGTCCCT
	GAATATATGC	CGCATGCTT	GAGTATGAG	TTGTCAGGG	TTGTCAGGG	TTGTCAGGG	TTGTCAGGG	TTGTCAGGG	TTGTCAGGG	TTGTCAGGG
21801	CGCGCTACT	CGGACGAC	AGTCCGACA	TTAGGAGCG	CACTCTCTT	CACTCTCTT	CACTCTCTT	CACTCTCTT	CACTCTCTT	CACTCTCTT
	CGCGATGAA	CGGATGCTG	TCAGGCTCT	ATTCCTGCG	GTGACGAA	GTGACGAA	GTGACGAA	GTGACGAA	GTGACGAA	GTGACGAA
21901	AGCAATATC	TTTTATTTT	ACACTCTG	GTGATATTT	ACCGGCTG	ACCGGCTG	ACCGGCTG	ACCGGCTG	ACCGGCTG	ACCGGCTG
	TCGTTTATC	AAATATACA	TTGAGAGCC	CACTATATA	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT	TTGAGTCT
22001	TCGCGCTAG	GCAGGACAC	GTTCGATAC	TTGATGTTG	TTGATGTTG	TTGATGTTG	TTGATGTTG	TTGATGTTG	TTGATGTTG	TTGATGTTG
	ACCGGCTAC	CGTCCCTG	CAACGCTAG	ACCAATATC	ACCAATATC	ACCAATATC	ACCAATATC	ACCAATATC	ACCAATATC	ACCAATATC
22101	GGCTGCGAC	CATACGAC	GGCTTTAGCA	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG
	CCGACGCTG	GTATGCTTG	CGCAATCTT	CCAGCGCGG	CCAGCGCGG	CCAGCGCGG	CCAGCGCGG	CCAGCGCGG	CCAGCGCGG	CCAGCGCGG
22201	GTTCGAGAC	TTGAACTATA	TCAGCGCGG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG	GTTCGCGCG
	CAAGCTGCT	ACCTTGTAT	AGTCCGCTC	CACCATGCT	CACCATGCT	CACCATGCT	CACCATGCT	CACCATGCT	CACCATGCT	CACCATGCT
22301	CCGACGCGG	TCAGCTTTG	TAGTCTGCT	CCCAAAAGG	CCCAAAAGG	CCCAAAAGG	CCCAAAAGG	CCCAAAAGG	CCCAAAAGG	CCCAAAAGG
	CGCTGCTCT	AGTTTAAAC	ATCGACGGA	GGTTTCTCC	GGTTTCTCC	GGTTTCTCC	GGTTTCTCC	GGTTTCTCC	GGTTTCTCC	GGTTTCTCC
22401	CGGCTGCGG	GTTCGATAC	AGCGCTTCA	TAAAGCTTT	GTTCGCTTA	GTTCGCTTA	GTTCGCTTA	GTTCGCTTA	GTTCGCTTA	GTTCGCTTA
	CCGACGCGG	CAATCTATG	TCGCGACCT	ATTTTCGGA	ATTTTCGGA	ATTTTCGGA	ATTTTCGGA	ATTTTCGGA	ATTTTCGGA	ATTTTCGGA
22501	GGCGGAAAC	TTATTTGCG	GTTCGCTAG	GTTCGCTAG	GTTCGCTAG	GTTCGCTAG	GTTCGCTAG	GTTCGCTAG	GTTCGCTAG	GTTCGCTAG
	CGGCTTTTG	ACTATACGG	CTCTCCGCG	CAGCAGTGC	CAGCAGTGC	CAGCAGTGC	CAGCAGTGC	CAGCAGTGC	CAGCAGTGC	CAGCAGTGC

Figure 15N

[illegible]

32/144

pmrkad5gag MER6B2

24201 CCTGCTCAA CCAAGTCCA AATATCTTTG AGGATTTTGG AGGATTTTGG CAAAGCTTTT CAAAGCTTTT CAAAGCTTTT CAAAGCTTTT CAAAGCTTTT
GAGAGGAGTT GTTTCAGGTT TTTTGAAGAC TCCAGAGATC TCCAGAGATC TCCAGAGATC TCCAGAGATC TCCAGAGATC TCCAGAGATC TCCAGAGATC

KpnI
24301 CTCTGAGTGG TTGTTGAGAC TCGAGGCTGA CAGAGGCTGG CTATGCTGAC TAAATGCTAG CAGAGGCTGG CAGAGGCTGG CAGAGGCTGG CAGAGGCTGG
GAGAGGCTGG AAGCAGCTTG AGCTCCCACT GTTTCAGGTT GATTCAGGTT GATTCAGGTT GATTCAGGTT GATTCAGGTT GATTCAGGTT GATTCAGGTT

24401 CCCCCAAGG TCAATGAGAC AGTATGAGT GATTCAGGTT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT
GAGAGGCTGG AGTATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT TCAATGAGT

24501 TACCCGAGT TCGCAGAGAG CAGTATGAG CAGTATGAG CAGTATGAG CAGTATGAG CAGTATGAG CAGTATGAG CAGTATGAG CAGTATGAG
ATGAGGCTGA ACCGCTGCTC GTTCAGGCTG CAGAGGCTGG CAGAGGCTGG CAGAGGCTGG CAGAGGCTGG CAGAGGCTGG CAGAGGCTGG

SphI
24601 TACGTTGAG CTGAGTGA TCGAGGCTTT CTGAGTGA TCGAGGCTTT CAGAGGCTTT CAGAGGCTTT CAGAGGCTTT CAGAGGCTTT CAGAGGCTTT
ATGAGGCTTC GAGTATGAGT AGCTCCCACT AGCTCCCACT AGCTCCCACT AGCTCCCACT AGCTCCCACT AGCTCCCACT AGCTCCCACT AGCTCCCACT

BglII
24701 CCGCAGGCTT CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

AclI
24801 CCGTCAAGG CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

PstI
24901 GAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

25001 GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

25101 AGGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

PstI
25201 CCGTCAAGG CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

KpnI
25301 ACCGCTGCTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

25401 CCGTCAAGG CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

25501 AGGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC CAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

25601 TTTTCTGAG GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC GAGAGGCTTC
GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT GAGTATGAGT

Figure 15P

pMRKad5gag MER682	
25701	GGGCGCTTGC TTCCGAGGAT GGCACCGAAA AAGAACTTTC AATTTTCTTC GTATCCACAG GACGAGGAGC AATACGTGGA CAATCAGGCA GATGAGTTT CCCGGAGACG AAGGGTCTTA CCGTGTGTTT TTCTTCGAGC TTCCAGGCGC GTATGCTTC CTGCTCTCC TTATGACCT GTACGTCCCT CTCTCCAA
25801	TGGACAGGCA GGAGGAGGAC ATGATCGAAG ACTGAGTAG GTATGAGTAG TAAAGTTTCC AGTATCGAGA GGTGTGAGAC GAACACCTT CACCTCCG ACCTGCTCTT CTCTCTCTTC TTACTTCTTC TGACCTCTTC GATCTGCTTC CTTCGAGGC TCACAGTCTG CTCTCTGCA GTCTCTGCA GTGAGGCTA
25901	CGCATTTCCC TGCCCGGCGC CCGAGAAATC GGCAGCTGGT TTCAACATTA GATCTCTCAG GTCGCCGCGG CACTGCCCGT TATGAGACCC GGTAAAGGCG ACGGCGCGCG GGTCTCTTAG CAGTTTGGCA AGTATCTAAC GATGTTTGAAG GGTGAGGATC GTGAGCGGCA ACGGCTGCG
26001	AACGCTAGAT GGGACACGAC TGGAAACGAG GCTCTGTAAT GTTCTGTGCG CCGCTCAAT CCGCTGCTTC TCTCTCTTAC CATCACGCG TGGTCTCCG TTGGCAATCA CCGTGTGCTG ACCTTGCTCC GCGCCATTCA GGTCTGTGCG CCGCTCAAT CCGCTGCTTC TCTCTCTTAC CATCACGCG TGGTCTCCG
26101	CGGCGGACAA GAGCGCATTA GTTCTGCTGCT TCGAGAGTGC TGAGGCAAC ATCTCTCTCG CCGCTCAAT CCGCTGCTTC TCTCTCTTAC CATCACGCG TGGTCTCCG CGGCGGCTTT CTTGCGCTAT CAACGAGCA AGTTCTGAGC ACCCTGCTTC TGAGGCAAC ATCTCTCTCG CCGCTCAAT CCGCTGCTTC TCTCTCTTAC CATCACGCG TGGTCTCCG
26201	CGGTACATTC CTGCTTACT ACCGTATCT CTACAGGCA TACTGACCG GCGGAGCGG CAGGACAGC AGCGGCGGA AGAGGAGTAT GTAGTCCGC ACCTCAAGG GGCATTTGAG GACATTAAGA TGCGAGTAG GATGCTCGGT ATGAGCTGCG CCGCTGCTTC TGAGGCAAC ATCTCTCTCG CCGCTCAAT CCGCTGCTTC TCTCTCTTAC CATCACGCG TGGTCTCCG
26301	TACGAGACT CTGACAAAGC CCAAGAAATC CACAGCGCG GTCTGCTGCT CAGGAGGAG GCTGCTCTG CATGAAATAC AGCGGCGGA CAGGAGGCA CAGGAGGCA ATCTCTCTGA GACTTTTTCG GTTCTTTAG GTCTGCGCG CAGGAGGAG GCTGCTCTG CATGAAATAC AGCGGCGGA CAGGAGGCA CAGGAGGCA CAGGAGGCA
26401	CTTAGAACA GATTTTTC GACTCTAT CTATATTC CAGAGAGAG GGCGGCTTC TTCTCTCTTC GTTCTGCTT CAGTAAATAC AGCGGCGGA CAGGAGGCA CAGGAGGCA GATCTCTTGT CTTAAAGAG GTAGAGACATA CCAATATTAAG TTCTCTCTTC CCGGCTTC GTTCTGCTT CAGTAAATAC AGCGGCGGA CAGGAGGCA CAGGAGGCA CAGGAGGCA
26501	CCCGCACTG CACTCTAT CACTCTAT ATCAGCTTC GCGTCTGCT TACTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG GGGCTGAG GGCATAGTG GTTCTGCTT TTTCTGCTT TACTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG
26601	CTAGTTTTCG GCGCTTTC AAATTAAGC GCGAAGCTA GCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG GATCAAGCG GCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG
26701	GAATTTCCA CCGCTTACT GTGGTTTAC CAGCCACAA TGCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CTTTAAGGCT GCGGAGTGA CAGCTCAATG GTGCGTCTT ACCTGAGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG
26801	GAACCTACAT GATATCTCCG GTCAACGAA TACGCGGCA CCGAAGCTA ATCTCTGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG CTGCGTCTGA CTATAGGCG CAGTCTCTT ATGCGCGGT GCGTCTGAG TAAAGGAGC TTCTCTCCG ATAAATGTTG TGTGAGCAT TATGAGTAT
26901	TGCGGCTGAG TGCGGCTG CCGTCTGTA CCGAGAAAT CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA AGGCGCATCA ACCGCGGAG GCGGACAT GGTCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA
27001	TCAGGCGCG AGCTTTCG CCGCTTCTG CACAGGTC CAGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG CCGTCTGAG AGTCTCTCG TCAAGCGCG GCGGAGGCA GTTCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA
27101	ACGAGTCTG GAGCTCTG CTTGCTCTG GTGCTGAG GATCTTTCAG ATGCGCGCG CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA TGTCTAGCCA CTGAGGAGC GAGGAGGAG CAGGCTGCTT CAGGCTGCTT CAGGCTGCTT CAGGCTGCTT CAGGCTGCTT CAGGCTGCTT CAGGCTGCTT CAGGCTGCTT
27201	TCTGAGAGC TGTCTCTG AGCGGCTG TGGAGCAT GGTATCTG GGTATCTG CAGGAGGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG AGAGCTCTG AGAGGAGC TCGCGCGAG ACCTCTGTA CTTTCTGAG TTAATTAAT CTTTCTGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG CAGGAGGAG

Figure 150

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27301 CTTCCCGCC ACTATCCGGA TCAATTATTT CTTAACTTTG AGGTGTTTAA GGAATGCGG GAGCGCTACG ACTGAATGTT AGTGGAGAG GAGAGCAAC
 GAGAGCCCG TGTATAGGCT AGTTAATATA GGAATGAAC TGGTATATT CTTGAGTTC CTTGAGTTC TACTATACG TACTATACG TACTATACG
 27401 TGGCCCTGAA ACACCTGCT CACTGCTGC GGCACAGTC CTTTCCCTC CTTTCCCTC CTTTCCCTC CTTTCCCTC CTTTCCCTC CTTTCCCTC
 ACAGGACTT TGTGACCAAG GTGACAGCG CGGTGTTTAC GAAATGCGG GAAATGCGG GAAATGCGG GAAATGCGG GAAATGCGG GAAATGCGG
 27501 CCGGCGCAC GCGTCCGCG TTACCGCCCA GGGAGAGCTT GCGGTAGTC TGAATGCGG GAAATGCGG GAAATGCGG GAAATGCGG GAAATGCGG
 GCGCGCGTG CCGAGCGCG AATGCGGCT CCGTCTCGA CCGGCGCT CCGGCGCT CCGGCGCT CCGGCGCT CCGGCGCT CCGGCGCT
 27601 CCGTCTGTC TCACTGTGAT TTGCACTGT CTTCACTGT CTTCACTGT CTTCACTGT CTTCACTGT CTTCACTGT CTTCACTGT CTTCACTGT
 GGGACACAG AGTACACTA AACGTGTACA GGAATGCGG CTTATGATG TCTAGAACG ACGGTAGAG CAGGACTCAT ATTATTTATG TCTTTATG
 27701 ATATACTGG GCTCTATCG CCATCTGTA AACGCCAG TCTTACCGG CCAAGCGAA CCAAGCGAA CCAAGCGAA CCAAGCGAA CCAAGCGAA
 TATATGACC CAGGATAGC GTTAGACAT TTGCGTGGC AGAATGCGG GGTTCGCTT GGTTCGCTT GGTTCGCTT GGTTCGCTT GGTTCGCTT
 27801 CTGTGATTTA CAACAGTTTC AACCCAGAG CAGTATGTC AGAATGAGG CTTCTGCTG GAGAGCTCG AGTGTATCG CATCATGTA TCTTACCTT
 GACACTAAT GTTGTCAAG TTGCGTCTG CTTCTGCTG TGTCTCTG GAGAGCTCG AGTGTATCG CATCATGTA TCTTACCTT TCTTACCTT
 27901 CCGGACCTT ACGATGCT CACCGCGCG TGTACACAG CTTACCGCT CTTACCGCT CTTACCGCT CTTACCGCT CTTACCGCT CTTACCGCT
 GCGGCTTCA TGTCTACCA TGTCTACCA TGTCTACCA TGTCTACCA TGTCTACCA TGTCTACCA TGTCTACCA TGTCTACCA TGTCTACCA
 28001 AACAGAGGT GAGCTTACCA AACCTTAGG GTATTAGCG AATGCGCG CTTACTGTC GTTATGAGC AATGCGCG CTTACTGTC GTTATGAGC
 TTGTCTTCA CTGGAATCT TTGGAATCC CATATCGG TTTCGCGTC GATGACACG CAATATCT TTATGTTCT TGAATGCGG GATGAGCT
 28101 TCAGCTTCT CTAGATGCG GGTGCGGTT ATTCTCTG TGTGATCT CTTATCTT ATACTACG TCTCTGCT AGGCTCGC GCTGCTT
 AGTCCAAAG GATCTTAGG CCAACCCCA TAAGAGACAG AACACTAGA TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG
 28201 TGCACATTT CATTTATCT CAGCTTTTA AACGCTGGG TGTGACCGA AGATGATG GATGATG GATGATG GATGATG GATGATG
 ACCTGTAAC GTAAATACA GTGGAATAT TTGCGACCC TTGCGACCC TTGCGACCC TTGCGACCC TTGCGACCC TTGCGACCC
 28301 GGTACCAAC AAAAGCTGA TTTAAGGAG CAGGCTGTA ATGTTACAT CCGAGCTGA GGTATGAT GGTATGAT GGTATGAT GGTATGAT
 CCAATGCGG TTTCACCT AAAATCTC GTTGGACAT TACATGTA TACATGTA TACATGTA TACATGTA TACATGTA
 28401 ATGAAAGCT GCTTATTCG CACAAACA AATTTGCA GTATCTGT TATGCTAT TATGCTAT TATGCTAT TATGCTAT TATGCTAT
 TACTTTTGA CCAATAAGCG GTGTTTTGT TTAAACCGT CATACGCA TTACGATGA TTACGATGA TTACGATGA TTACGATGA
 28501 CCAAGGTAA ATCAATAA CTTTATGTA TACTTTTCA TTTTATGA TGTGACAT TACCATGAC ATGAGCAAC AGTATAGTT GTGCCCCA
 GGTCCCATTT TCAGTATTT GAAATACAT ATGAAAGT AAATATCTT AAATATCTT AAATATCTT AAATATCTT AAATATCTT
 28601 CAATATGTT TGGAAACAG TGTGACTTC TGTGACTTC TGTGACTTC TGTGACTTC TGTGACTTC TGTGACTTC TGTGACTTC
 GTTTTACAC ACCTTTGTT ACCGTGAG CAGGCTGAC GATGATGTA ATGTCACAG CCAAGCGAG CATGATGA GATATATTT ATGTTTTT
 28701 GACGAGCTT TATTGAGGA AAGAAATGC CTTTATTTAC TAAGTTTAC ACTTATGTC ACCACTACT GCTTATGCT GTACCTACT CTATATTA
 CTGCTGCA ATACTCTT TTCTTTTAC GATTAATG ATTCATGT TGTGATGAC TGTGATGAC TGTGATGAC TGTGATGAC TGTGATGAC
 28801 AAAGTTAGC ATTAATTA GATAGGAT TAAACCCG GGTCTTTTC TGTCTATG TGTCTATG TGTCTATG TGTCTATG TGTCTATG
 TTTTCATCG TATATTAAT CTTATCTTA ATTGCGGG CCAATAGG AGGATGATG GTAGGAGG TGTGATGAC TGTGATGAC TGTGATGAC

Figure 15R

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28901 GCGCTACAAAC CTTGTAAGTCA GCTTCTCTGG ATGTACAGAT CTGACTTTGG CAGCAGCTCG TCCCGCGGAT TTCTTCCAGT CCAACTACAG CCAGCCAGTC
 CCGGNTCTTG GAACCTCACT CCGAAGACAC TACAGTCTGA GACTGAACCC GGTGGGTGAC ATGCGCGCTTA AACAGGTCA GGTGATGTGC CATTGGTATG
 29001 TAACAGAGAT GACCAACACA ACCAAGCGCG CCGCGCTTAC CCGACTTACA TTACACACAA ATACACCCCA AGTTCTGCG TTGTCANAT ACTGGGATMA
 ATTCTCTCTA CTGCTTGTGT TGGTTCGCGC GCGCGGATG GCTGATATCT AGATATGTTT TATGTTGGGT TCAAGACGG AACAGTTAT TGAACCTTAT
 29101 CTTGGGCATG TGGTGTCTCT CCGATGCTCT TATTTTGTGA TGTCTTATTA TGTCTTATTA TTAGTGTGCT CATCTCTGCG CTAAAGCGCA AACGCGCGCG ACCACCCATC
 GAACCCGTAC ACCACCAAGA GGTATCGCGA ATACAAACAT ACCGATATAT ATACACTTGA GTAGACGCG GTATTGCGGT TTGCGCGGCG TGGTATGAG
 29201 TATAGTCCCA TCATTGTGCT ACACCCAAAC ATATATATTA TGTATAGAT GTAGCGATTA ATACACATGT TCTTTCTCT TACAGTATGA TTAAATGAGA
 ATATCAAGGT AGTAAACAGA TGTGGTTTG TTAGTACTCT AGGTATCTTA CCGCGCGGAC TTGCTGTACA AGAAAGAGA ATGTATATCT ATTTTACTCT
 29301 CATGATCTCT CGAGTTTATA TATTACTGAC CTTTGTGCG CTTTGTGCG GGTGCTCCAC ATTGCGTGG GTTCTCACA TCGAAGTGA CTCGATTC A
 GTACTAAGGA GCTCAAAAT ATATGACTG GGAACAGCG GAAAGACAC GACAGGATG TACCGGACG CAAGAGTGT AGCTTATCT GACGTAGG T
 29401 GCGTTACAG TCTATTGCT TTACGGATTT GTACACCTCA CCGTCACTCT CAGCTTCAAT ACTGTGCTA TCGCTTTAT TCGCTTAT CCACTGCTT GACTGGCT
 CGAAGTGTCT AGATAAACGA ATGCGCTAA CAGTGGAGT GCGATGAGC GTCTGATGAG TCGACCCAGT AGCGAAATA GGTACCGTAA CTGACCCGTA
 29501 GTGTGCTCT TGCATATCTC AGACACCATC CCGAGTACAG GGACGAGCT ATAGCTGAGC TTTTATGAT TCTTTAATTA TGAATTTTAC TGTGCTTT
 CACAGCGGAA AGGTATAGAG TCTGTCTGAG GGGTATGTC CCGTCTCTGA TATGACTGCG AAGATCTTA AGAATTTAAT ACTTTAATG ACACGTAA
 29601 CTGCTGATTA TTGACGCT ATCTGCTTT TGTTCGCGA CCGTCAAGCG TCAAGACAT ATATCATGCG GATTCACCTG TATATGAT ATTCGAGT
 GACGACTAAT AACGTGGA TAGACCGAA ACAAGCGCT GAGGTCTCG AGTTCTGTA TATATGCT CTAAAGTGC ATATACCTTA TAAAGTTCAA
 29701 GCTACATGA AAAAGCGAT CTTTCCGAG CCGTGTATA TCGATCATC TCTGTTATG TGTCTTCCAG TACCATCTTA GCGCTAGCTA TATATCCIA
 CGATGTTACT TTTTGGCTA GAAAGCTTC GGACCAATAT AGTTAGTAG AGACAATACC ACNAGACGTC ATGATAGAT CGGATGAT ATATAGGAT
 29801 CCTTGCATT GGTGTGAGG CAATAGATCC CATGACCCAC CCAACTTTCC CCGCGCGCGG TATGCTTCCA CTGCAACAG TTGTTGCGCG CCGCTTTCTC
 GAACTGTAA CCGACCTTGC GTTATCTAG GTACTTGTG GTTGTAAAG GCGCGCGCGG ATACGAAGT GACGTTGTC AACAAAGCGG GCGGAAAT
 29901 CCAGCCCATC AGCTGCGCC ACCTTCTCC ACCCTCACTG AAATCAGCTA CTTTAAATCA ACAGAGGAG ATGACTGACA CCGTATATCT AGAAATGAC
 GGTGCTTGG TCGAGCGGG TCGAGAGCGG TGGGGTGC TTTAGTCTAT GAAATTAGAT TGTCTCTCTC TACTGACTGT GGGATCTAGA TCTTTACTG
 30001 GGAATTTATA CAGACGCG CCGCTAGAA AGACCGCGG CAGCGCGGA GCAACGCGC ATGAATCAAG AGCTCCAGA CATGTTTAC TTGACCAAGT
 CTTAATAT GTCTGCTCG GACGATCTT TCTGCGTCC GTGCGCGCT GTTGTGCG TACTTATTC TCGAGTTCT GTACCAATG AACCTGCTA
 30101 GCAAGCGG TATCTTTGT CTGTAAGC AGTCAAGT CACCTAGC AGTATACCA CCGGACCG CCGTATGCTAC AAGTTGCCA CCAAGCGT
 CCGTTTCCC ATAGAAGCA GAGCATTCG TCGGTTTCA CTGATGCTG TCAATATGT GCGCTGTGCG GGAATGATG TTCAAGCGT TCGCTTAT
 30201 GAATTTGTG GTCATGTG GAGAAAGCC CATTAACATA ACTCAGCACT GTTATTAAC CCAAGCGTGT CCAATGCTAC CTGTCAGG ACCTGAGAT
 CTTTACCAC CAGTACCAC CTCCTTTGCG GTATGCTAT TACTGCTGA GCACTCTTG GCTTCGAGG TAAATGATG GACAGTTTC TCGACTCTA
 30301 CTCCTGACCC TTATTAGAC CCGTGTGCT CTCAAGATC TTATCTCTT TAACTATTA AAAAATTA TAAAGTCA CTTACTTAAA ATCAGTTAGC
 GAGACGTGG NATATCTG GGACACGCCA GAGTTCTAG ATTGATATT TTTTATTATT ATTTGTTAGT GAAATGATTT TAGTCAATCG

Figure 155

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30401 AAATTTCTGT CCAGTTTATTT CAGCAGCACC TCCCTGTGCTT CCTCTCAGCT CTGTATATTC AGCTTCTCC TGGCTGCAAA CTCTCTCCAC AATCTAAATG
 TTTAAAGACA GGTCAATATA GTCTCTGTTC AGAACCGGGA GTAAAGTTCGA GACTATATACG TTGAAAGGAGG ACCGACGTTT GAAAGAGTGG TTAGATTTTAC
 30501 GAATGTCAUT TTCTCTCTGT TCCGTGTCAT CCGCACCAC TATCTTCATG TTCTTTCATG TGAAGGCGC AATACGCTCT GAGATACCT TCAACCCCTT
 CTTACAGTCA AAGGAGGACA AGGATAGGTA GTCTGTGATG ATAGAAATAT ACACAGCTCT ACTTCTGCGG TTCTGCGAGA CTCTATATGA AGTTGCGG A
 30601 GTATCCATAT GACAGGAAA CCGGTCTCTC ACTGTCTCTT TTCTTACTC CTCTCTTGT ATCTCCCAT GTGTTTCAG AGATCTCCCC TGGGTACT :
 CATAGATATA CTGTGCTTT GCGCAGGAGG TTGACAGCGA TTAGGAGTGA GAGGGAACA TAGCGGTTA CCAAAAGTTC TCTCAGAGGAG ACCCCATGAG
 30701 TCTTTGCGCC TATCCGAACC TCTAGTTTACC TCCATATGGA TGTCTTGGCT CAATATGAGC AACTGTCTCT CTCTGACGA GCGCGGCGAC CTTACTCTCT:
 AGAACGCGG ATAGGCTTGG AGATCAATGG AGCTTACCCT ATGACGCGTA GTTTTACCAG TTGCGGAGA GAGACCTCTT CCGGCGCTTG GANTGAGI I
 30801 AAATGTNAC CACTGTGAGC CCAGCTCTCA AAAAACCMA GTTAAATATA AACTCGMAA TATCTGACC CCTCACGTTT ACCTCAGAGG CCTTAACTCT
 TTTTACATTT GTACACTTGG GTGTGAGAT TTTTGTGAT CAGTTGTAT TTGACCTTTT ATGAGCGTGG GAGGTGTCMA TGGAGTCTTC GGTATGACA
 30901 GGTGCGGCC GCACCTCTTA TGTGCGCGG CAACACATC ACATTCAT ACAGGCCCC GCTAACCGTG CAGGACTCCA AACTTACGAT TGGCACCCNA
 CCGACGCGCG CGTGAGAT ACCAGCGGCC GTTGTGTGAG TGTAGCTTA GTGTGCGG CCAATGCGAC TGTCTGAGT TTGAATGTTA AGGTGGGT I
 31001 GGAACCCCTCA CAGTGTGAGA AGGAAGCTA GCGCTGCAA CATCAGGCC CCGTACCCAC ATACGCTTAC GTACGCTTAC TATCACTGCC TACACCCCTT
 CCGCGGAGT GTACAGCTCT TCTTTGTGAT CCGGACGTTT GTATGCGCGG GAGGTGTGG TGGCTATCT CATGGAATG ATATGACGAG AGTGGGGA
 31101 TAACTACTGC CACTGTGAGC TTGGCATTTG ACTGTGAGA GCGCATTTAT ACACAAATG GAATACAGG ACTTAACTAG GCGGCTCTCT TGCATGTA
 ATTGATGAG GTGACCATTC TGAICTTCT CCGTAAATTA TGTGTTTAC CTTTGATCC TGAATTCATG CCGCGAGGA ACCTACATTT
 31201 AGACGACTTA ACACCTTGA CCGTAGAAC TGTGCGAT GCACTATTA ATATATCTC CTTCGAACT AAGCTTGGG GAGCTTGGG TTTTATTTA
 TCTGTGAT TGTGAACT TGTGAGCTT ACCAGCTCA CACTGTTA TATTATGAG GAACCTTGA TTTCATGAC GATCGAACC ANACTAT
 31301 CAAGGCAATA TGCACCTTAA TGTAGCAGGA GCACTAAGGA TTGATTTCA AACAGACCG CTTATCTTGA ATGTTATGTA TCCCTTGTAT GCTCAAAAC
 GTTCCCTTAT ACCTGATTT ACATGCTCT CCGATCTCT AACTAGAGT TTGTCTCG GAATATGAC TACATCAAT AGGCAACTA CGATTTT
 31401 AACTAATCT AGACTAGGA CAGGCGCTC TTTTATATA CTCAGGCC ACCTTGATA TTACTACGA CAAAGGCTT TACTTTTGA CAGCTTCA
 TGTATTTAGA TCTGATCT GTCCCGGAG AAAATATTT GAGTGGTG TTGAACCTAT TATGATGTT GTTTCCGAA ATGAACAAAT GTCCAAATTT
 31501 CAATTCAAA AGCTTGAAG TTAACTAAG CACTGCCAAG GGTGTGAT TTGACCTTAC AGCATAGC ATTAATGCG AGATGGCT TGAATTTGT
 GTTANGGTT TTGAACTCC AATTCATTC GTGACGTTT CCGACTACA AACTCGATG TCGATATCG TAAATACGTC CTCTACCGGA ACTTAAAGCA
 31601 TCACTTAATG CAGCAACAC AAATCCCTC AAAACAAA TTGTGATGG CCTAGATGG GATTCANACA AGGCTATGG TTCTAAACTA GGAATCTGCT
 AUTGTATTTAC GTGTTTGTG TTTAGGGAG TTTTGTGTTT AACCGTACC GATCTTAA CTAGCTTTCT TCCGATACCA AGATTTGAT CTTGACCGW
 31701 TTAGTTTGA CAGCAGAT GCAATPACAG TAGGAACAA AATATGAT AGCTAATCT TCTGACCCAC ACCAGTCCA TCTCTTACT GTACACTAA
 AATCAAACT GTCTGTCCA CCGTATGTC ATCTTTGT TTTTATTTA TTGATTTGA ACACCTGAG TGGTGGAGT AAGGATTTGA CATCTGATTT
 31801 TGCAGAGAAA GATGTAAAC TCACTTGGT CTTAACAAA TGTGACATC AATATCTTC TACACTTTGA GTTTTGGCTG TTAAGGCGAG TTTGCTGCTA
 ACCTCTCTT CTAGATTTG AGTGAACCA GAATGTTTT ACACCTGAG TTTATGAGG ATGTCAAGT CAAAAGCGC AATTTCCGTC AAACCGAGT
 31901 ATATCTGAAA CAGTTCMAAG TGTCACTCT ATTAATGAT TTGAGGATA TGTAGGCTA CTAAACAA CTCTCTCTG CCGCAATAT TTAACTTTA
 TATAGACCTT GTCAAGTTTC ACAGTAGAA TATATTTCTA AACTGCTTTT ACCTACCAT CATTTGTTAA GGAAGGACCT GGTCTTTATA ACCTTAAAT
 32001 GAAATGAGA TCTTACTGAA GGCACAGCT ATACAAACC TCTTGTGATTT ATGCTTACC TATCAGCTTA TCCAAATCT CAGCTTAAA CTGCGAAAG
 CTTTACCTCT AGATGACTT CCGTGTGGA TATGTTTGGG ACMACTAAA TACGGAATG ATAGTCAAT AGCTTTTGA GTGCCATTTT GAGCTTTTCT

Figure 15T

PHKAD591g MER682

32101 TACATATGTC AGTCAGATTT ACTTAAACGG AGCAAAACT AAACCTGTAA CACTAACAT TACACTAAC GGTACACAG AMCAGGAGA CACAATCTCA
ATTGTAAACAG TCAGTTCAAA TGAATTTGCC TCCTTTTGA TTCTGACAT GTATGTGTA ATGTGATTTG CCATGTGTCC TTGTCTCTCT GTGTGAGT

32201 AGTGCATACT CTATGTCAAT TTCAATGGAC TCTCTGTCT ACATATAT TATGTGAATA TTCTGCAT CCTTTACAC TTTTTCATAC ATTGCCCAN
TCACGTATGA GATACAGTAA AGTATCCCTG ACCAGACCGG TCTTATATTA ATTACTTTAT AACCTGTGA GAGAAATGTG AAAAAGTATG TAACGRTT

32301 AATAAGAAAT CGTTGTGTTT ATGTTTCMAC GTCTTTTATTT TGCATTTGA GAAATTTGA ATTCAATTTT CATTGACGTAG TATAGCCCCA CCATCCACATA
TTATTTCTTA GCAAAACACA TACAAGTTG CACAATATA AAGTTAACT CTTTAAACT TONTAATAA GTACGTATC ATATCGGGGT GATGCTGTGA
GCTATATACAG ATACCGGTAC CTTTATCAAA CTCACAGAAC CCACTATATC AACCTTCAC CTCCTCTCCA TGTCTCTCTC ATGTCTCTAG AMAGGG

32401 GGNATATGTC TAGTGGCATG GAATTAATTT GAGTGTCTTG GATCATTAAG TTATATTTCA CACGCTTTC TGTCTGAGCA AACGCTCAT AGTAAAT
GCTGGCTTA AAAGCATCA TATCATGGT MACACATA TTCTCTGTAT AAATATCCAC TATATAGGT GTGCCAAGG ACAGCTGGT TTGCGAGTAG TCATATAT
CGACCGAAT TTTCGTAGT ATAGTACCA TTGTCTGTAT ATATATAGGT GTGCCAAGG ACAGCTGGT TTGCGAGTAG TCATATAT

32501 GCTGGCTTA AAAGCATCA TATCATGGT MACACATA TTCTCTGTAT AAATATCCAC TATATAGGT GTGCCAAGG ACAGCTGGT TTGCGAGTAG TCATATAT

32601 ATAAACTCCC CCGGACGTC ACTTAAGTTC ATGTCTCT ATGTCTCT ATGTCTCT ATGTCTCT ATGTCTCT ATGTCTCT ATGTCTCT ATGTCTCT ATGTCTCT
TATTTGAGGG GCGGCTGAG TGAATTCAG TACAGGACA GTCTCAGCAC TCGGTGTCTG AGGACAGGTT GAACGCCAAC GAATTTGCCG CGGCTTCT

32701 AAGTCCACGC CTACATGGGG GTAGATCAT. AATGTGCAAT CAGGATAGGG CGTGTGTCT GCAGAGGCG GCGATTAAC TGTGTGCCGC GCGCTTCTCT
TTGAGGTGCG GATGTACCCC GATCTCAGTA TTGACAGCTA GTCTATCTCC GCCACCGCA CGTGTGTGCC GCTTATTTG ACAGCGGG CGGCGAGCA

32801 CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA CTTGAGGAA
GAGCTGCTT ATGTTGTACC GTACACAGAG GAGTGTCTAC TAAGCTGTAC TAAGCTGTAC TAAGCTGTAC TAAGCTGTAC TAAGCTGTAC TAAGCTGTAC TAAGCTGTAC

32901 TCACCTTAAT CAGCAGTA ACTGACGAC AGCAGCAGCA TATTTTCTAA AATCCACAG TCGAATGGCG TGTATCCAAA GCTCATGGCG GGAACACAG
AGTGAATTTA GTGTGTCTAT TCACTGTCTG TCTGTGTCT ATACAAATTT TTAGGTGTAC AGTGTCTGCG ACATAGCTTT CCGGTACCG CCGCTGTGTC

33001 AACCCAGCTG GCGATCATAC CACAAGGCA GGTAGATTAA GTGCGACCC CTCATAAACA CACTGTGACAT AACATTTACC TCTTTTGGCA TTTTCTAAT
TTGGGTGCAC GGTAGATATG GTTTTGGCT CCATCTAAT CACCGCTGG GAGTATTTCT GCGACCTGTA TTGTAAATGG AGAAGACCTT ACACATTTAA

33101 CACACCTCC CCGTACCTTA TAACTCTG ATTAACATG GCGCATCA CACATCTT NAACCATCT GCGAAGCT GTCCGCGCG TATACACTTA
GTGTGTGAGG GCGATGTAT ATTGAGAC TAATTTGTAC CCGGTAGGT GTGTGTAGCA TTGTGTGAC CCGTTTGGG CCGGCGCGCG ATATGTGAC

33201 AGGAAACCGG GACTGAAACA ATGACAGTGG AGAGCCGAG ACTGTANCC TGTGGGTCC TACTGTAGT TACGTAGT TACGTAGT TACGTAGT TACGTAGT TACGTAGT
TCCCTTGGCC CTGACCTTGT TACTGTGACC TGTGGGTCC TGTGGGTCC TGTGGGTCC TGTGGGTCC TGTGGGTCC TGTGGGTCC TGTGGGTCC TGTGGGTCC

33301 CTTGCTACCA CTTGCTCAGG ATTACAGCT CTTGCTCAGG TACAGCATA TCCAGGGA CAGCCATTC CTGATCAGC GTAAATCCCA CACTTCAGCG
GCGATATGT GAGAGCTCC TAATTTGGA GAGGCGCA ATCTGTGTAT AGGCTGCTT GTGTGTGTAG GACTGTAGT CATTTAGGCT GTGACCTC

33401 AGACCTGCG ACCTACTCA GGTGTGCTAT TGTCAAGTG GTACATCTGG GAGAGCTGT ATGATCTCC TGTGTGTAG AGTATGTAG CCGGTGTTC TGTCTCAA
TTCTGAGCG TGCATTTGAT GCAACAGCTA ACATTTTAC ACTGTATGCC CACTGTGCT TACTGTGCT TACTGTGCT TACTGTGCT TACTGTGCT TACTGTGCT

33501 GAGGTAGAC GATCCCTACT GTAGGATG GCGGATGCA CCGGCTCTCT TGGCTGTAG ACAGCCAGCA TCACAGTAG GTTTACCTTG CCGGCTGCT CATATATTA
CCTCCATCTG CTAGGATGA CATGCCCTAC CCGGCTCTCT TGGCTGTAG ACAGCCAGCA TCACAGTAG GTTTACCTTG CCGGCTGCT CATATATTA

Figure 15U

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33601	CTGTAAACAAA ACCAGGTGCG GCGGTGACAA ACAGATCTGC GTCTCTGGTC TTGCTGCTTA GATGCTCTG TGTAGTAGTT GTATTATATC CACTCTCTTA
33701	GACTTCTGTT TGTTCACGCG CGGCTGCTG GCTTCGGGTT CTATCTTAAC TCTTCTATGC GCTCTCTATC TGTATACCTA GATTAAGCCA CACCCAGCT/
33801	ATGCTAGGTC CGCGGGGAC CGAAGCCCA GATACATTTG AGCATATAGT GCGGACGCG ACTATGTAG GTGGTGGCTT CTATTGCGT GTGGCTCG/
33901	ACCTACACAT TGTCTCTGCG AGTCACACAC GGAAGAGCGT GGAAGACCTG GATTAATTTT TTATTCCAAA AGATTATCCA AAGCTTANA
34001	TGATATGTA AGCAAGACGC TCAGTGTGTG CCGTCTCTGC CTTCTCTGAC CTTCTTATGTA CAAAAAANA AATAAGTTT TCTATATAGT TTGCGNGTTT
34101	ATGAATCT ATTAAGTGAA CCGCTCTCC TCGGTGTGCG TTGCTTAACT CTACAGCCA AGACAGATA ATGCCATTTG TAAGATGTTG CACATGCT/
34201	TACTTCTAGA TAATTCACIT GCGCGAGCG AGCGCAACCG ACCAGTTTCA GATGTGGTT TCTGTCTAT TACCGTAAAC ATTCTACAC GTGTACCG/
34301	TCCAAAAGGC AAGGCGGCT CAGCTCCAG TCGACGTAA GCTTAAACCC TTGAGGTGA ATCTCTCTA TAACATTTCC AGCATTCCA ACCATGCCA
34401	AGGTTTTCG TTGCGCGGGA GTGCAAGTTC ACTGCTATG TATCTCTAAG CAATTCGCG TATATTTGAG GCGGTAAACA TTTTGTAGCG AGGTCTCGG
34501	ATTAATTTCT ATCTGCCAC CTCTCTANTA CTCTCAGGTT CCTCAGAGC CTGTATAGTA TTCTANAGCG GAACATTAA TTTTGTAGCG AGGTCTCGG
34601	TTATTAGAG TAGAGCGTG GAAGAGTTAT ATAGAGTTTC GTTTAGGCT TATATTTGAG GCGGTAAACA TTTTGTAGCG AGGTCTCGG
34701	CAOCTCTCAG CAGCGAATCA TGAATCCAAA AATTCAAGTT CCTCAGAGC CTGTATAGTA TTCTANAGCG GAACATTAA TTTTGTAGCG AGGTCTCGG
34801	GTGCGAGTTC GTGCTTNGT ACTAACGTTT TTAAGTCCAA GAGGTGTCTG GACATATTTG AATTTTCTG CTTTCTCTG AGATTATAGG CACACTGAT
34901	GTGCGAGTTC GTGCTTNGT ACTAACGTTT TTAAGTCCAA GAGGTGTCTG GACATATTTG AATTTTCTG CTTTCTCTG AGATTATAGG CACACTGAT
35001	GTGCGAGTTC GTGCTTNGT ACTAACGTTT TTAAGTCCAA GAGGTGTCTG GACATATTTG AATTTTCTG CTTTCTCTG AGATTATAGG CACACTGAT
35101	GTGCGAGTTC GTGCTTNGT ACTAACGTTT TTAAGTCCAA GAGGTGTCTG GACATATTTG AATTTTCTG CTTTCTCTG AGATTATAGG CACACTGAT
35201	GTGCGAGTTC GTGCTTNGT ACTAACGTTT TTAAGTCCAA GAGGTGTCTG GACATATTTG AATTTTCTG CTTTCTCTG AGATTATAGG CACACTGAT

Figure 1SV

pmrkad599 MER682

35301	CAATTTTACGA AACTACAT TCCACACCA TACAGTTAC TCCGCTTAA AACCTAGTC ACCGCTCCG TTCCACACCC CCGCCCAACG TCACAACTC	GTAAATTTCT TTGTATGTTA AGGGTTGCT ATGTTCATG ACCGCTGAT TTGGATGAG TGGGCGGCG AGGGTGGCG GGGCGGTGC AGTGTTCAG
35401	CACGCCCTCA TTATCATAT TGCCTCAAT CAAATTAAG TATATTTATG ATATCTTAA TTAAATTTT GATCTTCCA GCGAGGCTG GATGCCCTT	GTGGGGAGT AATAGTATA CCGAGTTAG GTTTTATTC ATATATATG TACTACATAT TACTTATG CCTACACCT GCGCTCCGAC CTACCGAAG
35501	CCCATTTTGA TTCTTCTCC TTCCGCGGC ATCGGNTG CCGCTTGA GCGCTTGA GCGCTTGA GCGCTTGA GCGCTTGA GCGCTTGA	GGTAAATCT MAGAGAGCG AAGCGCGCG TAGCCCTAG GCGCAACTT CCGCTTGA GCGCTTGA GCGCTTGA GCGCTTGA GCGCTTGA
35601	CGGTCGCTTT GCGTCAGAC CATTAAAGG CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	CGGTCAGAC CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
35701	GAAGTGGGGA AAGTGGGGA GACTATNAG ATACAGGCG TTTCGCTCT GAGCTTCTT GAGCTTCTT GAGCTTCTT GAGCTTCTT GAGCTTCTT	CTCCACCGCT TTGGCTGTC CTGATATTT TATGCTCCG AAGTGGGGA TTTCGCTCT GAGCTTCTT GAGCTTCTT GAGCTTCTT GAGCTTCTT
35801	CTGTCCGCT TTCTCCCTT GGGAGGCTG GCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	GACAGCGGA AAGAGGAG CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
35901	TGCACCAACC CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	AGCTGCTTGG GCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36001	CACCTGTNAC AGATTTAGCA GAGGAGGTA TGTAGGCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	GTGACCACTG TCTTAATCT TTCTGCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36101	ATCTGCGCTC TGTGAGGCG AATTACCTT GGAAGAGAG TTGTGCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	TAGAGCGGAG ACGCTTCTT GGAAGAGAG TTGTGCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36201	ACGCGCAGT TACGCGGCA AAGAGAGAT CTCAAGAGA TCCCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	TCTGCTCTA ATCGCGCTT TTTTCTCTA GAGTCTTCT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36301	TTTGTGCTAG AGATTATCA AAGGATCTT CACTTATAT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	AAACCACTAC TCTAATAGT TTCTCTAGA GTGATCTAG GAAATTTAG TTAGATTTCA TATATCTCA TTGAGGCGA ACTGTCAATG GTTACONAT
36401	TCAGTGAAGC ACCTATCTA GCGCTGCTT TATTTGCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	TGATACTCCG TGGATAGAGT CCGTACAGAG ATNAGAGAG CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36501	TGGCGCCAGT GCTGCAATGA TACCGCGAG CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	ACCGCGTCA CCGCTTCTT ATGCGCTCT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36601	CGTCAACTT TATCCGCTT CATTCAATCT ATTAATTTT GCGCGAGAG CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	GGAGCTTGA ATAGCGGAG GTAGGTAGA TATTAACAA CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36701	CTACAGCAT CTGTGTGCA CCGCTTCTT TGTGTAGC TTCACTAGC TCCGCTTCT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	GATGTCCGTA GCAACAGT GCGAGGAGT AACTATCCG AAGTATGTC AGGCCAAGG TTGCTAGTTC CCGCTTCTT ACTAGGGGT ACAACAGT
36801	AAAGCGGTT AGCTCTCTG GTCTCTCAT CCGTCTAGA AGTATGTC CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	TTTTCGCAA TCGAGGAGC CAGGAGCTA GCAAGATCT TCAATCAAC CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
36901	GTCAAGCAT CCGTAAATG CTTTCTGTC AGCTGAGT ACTTAACCA CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT	CAGTACGTA GCGATTTCT GCAAGAGC TGAAGTGGT CACTAAGACT CTTATCAGAT ACGCGCTCG CTCACAGAG ACGCGCGCA

Figure 15W

PMRKA15gag MERG82

37001 CACACCGGA TATACCGCG CCACATATCA GAACTTTAA ACTGCTCATC ATTCGAAAC GTCTCTCGG CGGAAACTC TCAAGGATCT TACTCTCTT
 GTTGTGCGCT ATTATGCGCG GGTGTATCTT CTGGAATTT TCAAGGATG TACCTTTTG CAGCAGGCC CGCTTTGAG AGTCCCTAGA ATGCGACAA
 GAGATCCAGT TCGATTTAAC CCACTCGTC ACCTAATCTA TCTTCAGCAT CTCTTACTT CACCAAGCTT TCTGGTGAG CAAACACAGG AAGCCAAAT
 CTCAGGTCA AGCTACATTC GGTGAGCAGT TCGCTTCACT AGAATCTCTA GAAATGAAA GTCTGCGCA AGCCCACTC GTTTTGTCC TTCCGTTTIA
 GCGCGAAAA AGGAAATAG GCGGACACG AATGCTTGA TACTATACT TTCTCTTTT CAAATTAAT GAGCATTTA TCAGGTTAT TCTCTCATTA
 CCGCGTTTT TCCCTTATC CCGCTTTCG TTACAACTT ATGATATCTA GAGGAAAA GTTATAATA CTTCGTAAT AGTCCCAATA ACAGAGTAA
 GCGGATACAT ATTGATGT ATTATGAAA ATAAACAAAT AGGATTTTG CTACATTTT CCGGAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAA
 GCGCTATGTA TAACTTACA TAAATCTTT TATTGTTTA TCCCGNAGC GCGTGTAAAG GCGCTTTTCA CGGTGAGCTG CAGATCTCTT GGTAAATAATA

37101

37201

37301

37401 CATGACATTA ACCTATAAA ATAGCGGTAT CACGAGGCC TTCTGCTTTC AGTAATGGA TTGGAATCT TAAT (SEQ ID NO: 27)
 GTACTGTAAAT TGATATTTT TATCCGATA GTGCTCGCG AAGCAGAG TTCTTACCT AGGCTTANGA ATTA (SEQ ID NO: 28)

Figure 15X

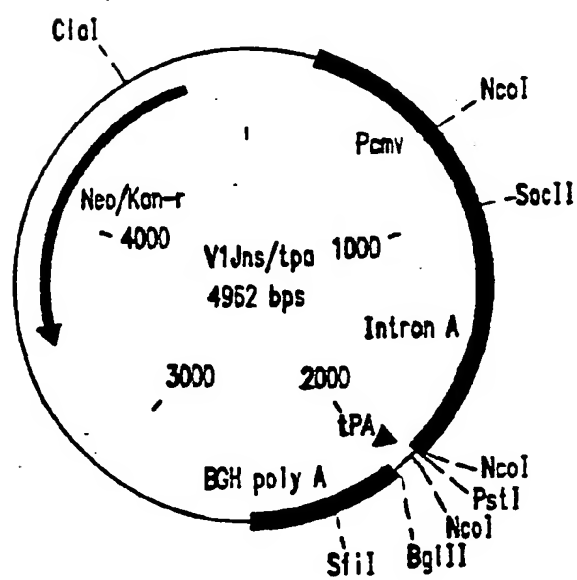
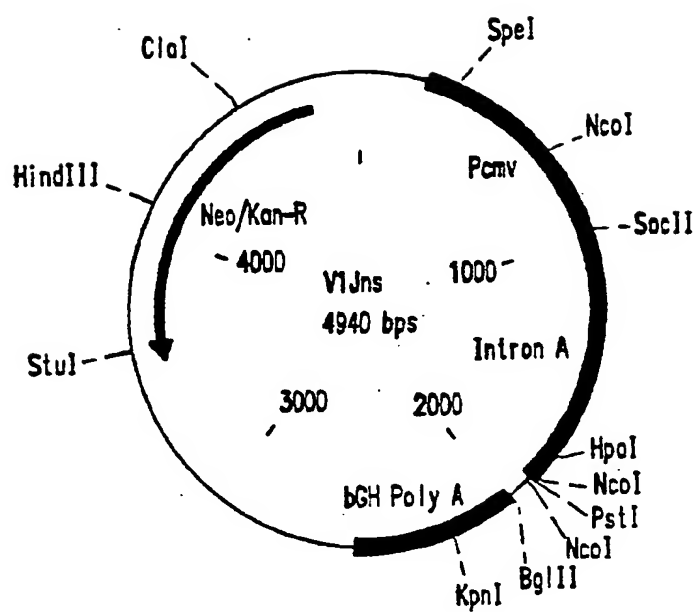


FIGURE 16

AGATCTACCATGGCCCCATCTCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGTGAA
 BgIII MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLys
 1 10 20

GCAGTGGCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL
 30 40 50

AGATTGGCCCGAGAACCCTACAACACCCTGTGTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal
 60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLys
 80 90 100

GAAGAACTCTGTGACTGTGCTGGCTGTGGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG
 sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA
 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC
 loPheTnrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly
 140 150

TCCCCTGCCATCTTCCAGTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGln
 160 170 180

GTACATGGCTGCCCTGTATGTGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL
 190 200 210

TGCTGAGGTGGGGCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGGCTATGAGCTGCAC
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis
 220 230

CCGACAAGTGGACTGTGCAGCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGln
 240 250 260

CAAGCTGAAGTGGGCTCCCAAATCTACCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL
 270 280 290

FIGURE 17A

TGA CTGAGGTGATCCCGCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGAGATCCTGAAGGAGCCTGTGCAT
 EüThrGluVolIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProVolHis
 300 310

GGGGTGACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAATCTA
 GlyVolTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGCCACACCAATGATGTGAAGCAGCTGA
 rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMeLArgGlyAlaHisThrAsnAspVolLysGlnLeuT
 350 360 370

CTCAGGCTGTGCAGAAGATCACCCTGAGTCCATTGTGATCTGGGGCAAGACCCCAAGTTCAAGCTGCCCATCCAGAAG
 hrGluAlaVolGlnLysIleThrThrGluSerIleVolIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT
 GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheVolAsnThrProProLe
 400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG
 uVolLysLeuTrpTyrGlnLeuGluLysGluProIleVolGlyAlaGluThrPheTyrVolAlaGlyAlaAlaAsnArgG
 430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGCAGGCAGAAGTGGTGACCCCTGACTGACACCAACCAAG
 luThrLysLeuGlyLysAlaGlyTyrVolThrAsnArgGlyArgGlnLysVolVolThrLeuThrAspThrThrAsnGln
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC
 LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluVolAsnIleVolThrAlaSerGlnTyrAl
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACAGATCATTGAGCAGCTGATCAAGAAGG
 aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuVolAsnGlnIleIleGluGlnLeuIleLysLysG
 510 520 530

AGAAGGTGTACCTGGCCTGGTGCCTGCCACAAGGCATTGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
 luLysVolTyrLeuAlaTrpVolProAlaHisLysGlyIleGlyGlyAsnGluGlnVolAspLysLeuVolSerAlaGly
 540 550

ATCAGGAAGGTGCTGTTCCCTGGATGGCATTGACAAGGCCAGGATGAGCATGAGAAGTACCACTCCAAGTGGAGGGCTAT
 lleArgLysVolLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe
 560 570 580

FIGURE 17B

GGCTCTGACTTCAACCTGCCCTGTGGTGGCTAAGGAGATTGTGGCTCCTGTGACAAGTGCAGCTGAAGGGGAGG
 tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA
 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCCTGCACCCACCTGGAGGGCAAGGTGATCCTGGT
 lαMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal
 620 630

GCTGTGCATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCTCTGT
 AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe
 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAATTCACTGGGGCCACAGTGAGGGCTG
 uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA
 670 680 690

CCTGCTGGTGGCTGGCATCAAGCAGGAGTTTGGCATCCCTACAACCCCACTCCACGGGGTGGTGGCTCCATGAAC
 lαCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn
 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT
 LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle
 720 730 740

CCACAACCTTCAAGAGGAAGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
 eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG
 750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCAGCAAGATCCAGAACTTCAGGGTGTACTACAGGACTCCAGGAACCCCTGTGG
 lαThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp
 780 790

AAGGCCCTGCCAAGCTGCTGTGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACCTGTGACATCAAGGTGGTGGCCAG
 LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr
 800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT
 gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx
 830 840 850

AAAGCCCGGCAGATCT (SEQ ID NO: 3)
 Xx Bg11 (SEQ ID NO: 4)

FIGURE 17C

FIGURE 18

WT	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT	-42
OPT	- ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC	
	M G G K W S K R S V P G W S	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT	-84
OPT	- ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC	
	T V R E R M R R A E P A A D	-28
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA	-126
OPT	- AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC	
	R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	- GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC	
	V S R D L E K H G A I T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC	
	N T A A T N A D C A W L E A	-70
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	- CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG	
	Q E D E E V G F P V R P Q V	-84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC	
	P L R P M T Y K G A V D L S	-98
WT	- CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC	
	H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC	
	S Q K R Q D I L D L W V Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC	
	T Q G Y F P D W Q N Y T P G	-140

FIGURE 19A

WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG	-462
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG	
	P G I R F P L T F G W C F K	-154
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA	-504
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG	
	L V P V E P E K V E E A N E	-168
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG	-546
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC	
	G E N N C L L H P M S Q H G	-182
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC	-588
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC	
	I E D P E K E V L E W R F D	-196
WT	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG	-630
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC	
	S K L A F H H V A R E L H P	-210
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)	-651
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9)	
	E Y Y K D C (SEQ ID NO:10)	-216

FIGURE 19B

V1Jns/nef *PstI* *BglIII*
 CATGGGTCCTTTTCIGGAGTCACCGTCCTTGGAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC
 M G G K W S K R S V P
 CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGACAGATCTGCTGTGTCCTTCTAGTTGCCAGC (SEQ ID NO: 38)
 H P E Y Y K D C * (contained within SEQ ID NO: 10)
SrfI *BglIII*

V1Jns/nef(G2A.LLAA)
PstI *BglIII*
 CATGGGTCCTTTTCIGGAGTCACCGTCCTTGGAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC
 M A G K W S K R S V P
 CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGACAGATCTGCTGTGTCCTTCTAGTTGCCAGC (SEQ ID NO: 39)
 H P E Y Y K D C * (contained within SEQ ID NO: 14)
SrfI *BglIII*

V1Jns/tpanef & V1Jns/tpanef(LLAA)
PstI *BglIII*
 CATGGGTCCTTTTCIGGAGTCACCGTCCTTATATCTAGATCACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG
 M D A M K R G L C V
 CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG ATC TCC TCC AAG AGG TCC GTG CCC
 L L L C G A V F V S P S E I S S K R S V P
BglIII
 CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGACAGATCTGCTGTGTCCTTCTAGTTGCCAGC (SEQ ID NO: 40)
 H P E Y Y K D C * (contained within SEQ ID NO: 16)
SrfI *BglIII*

FIGURE 20

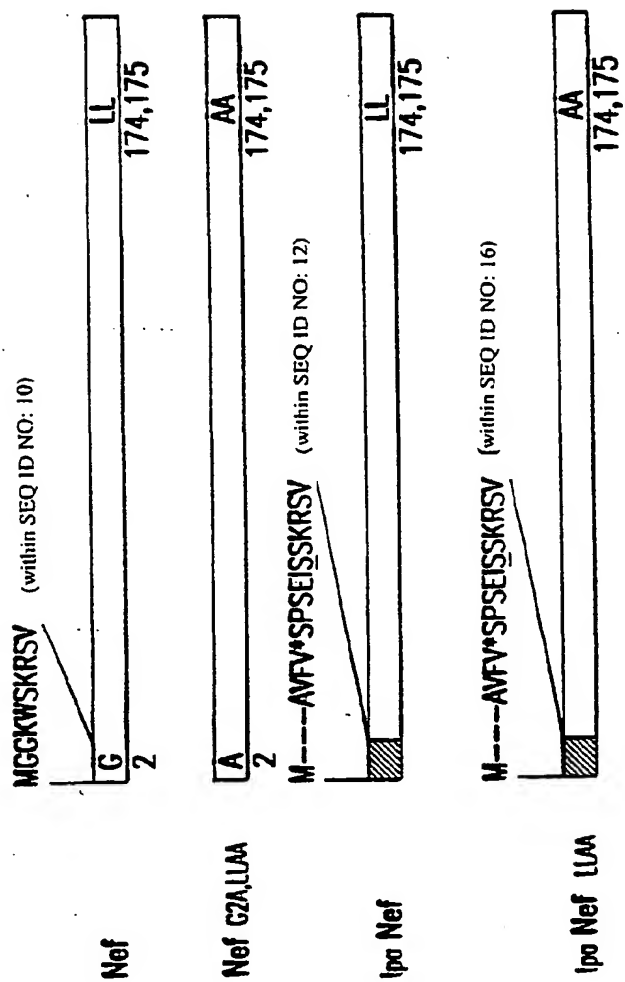


FIGURE 21

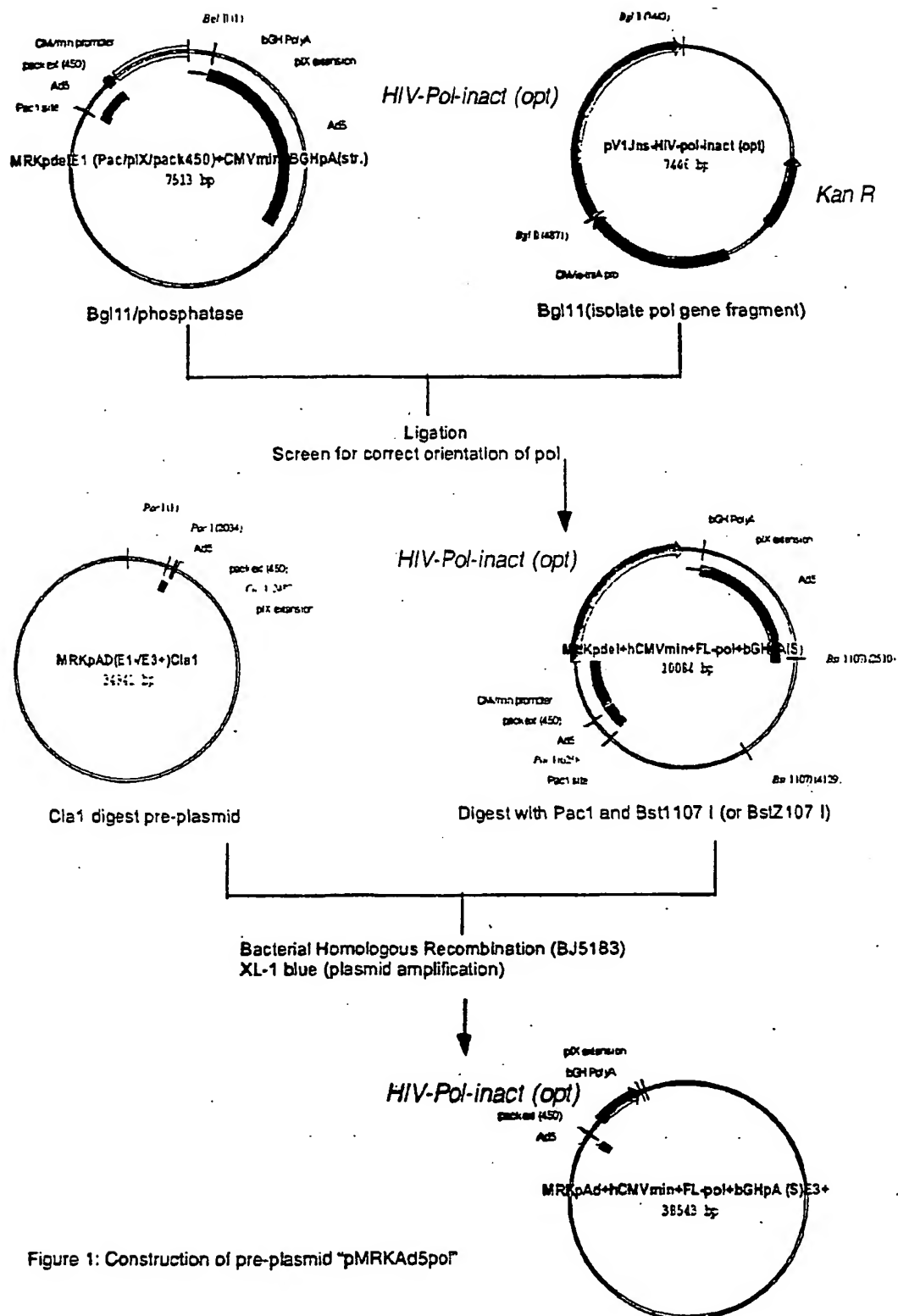


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22

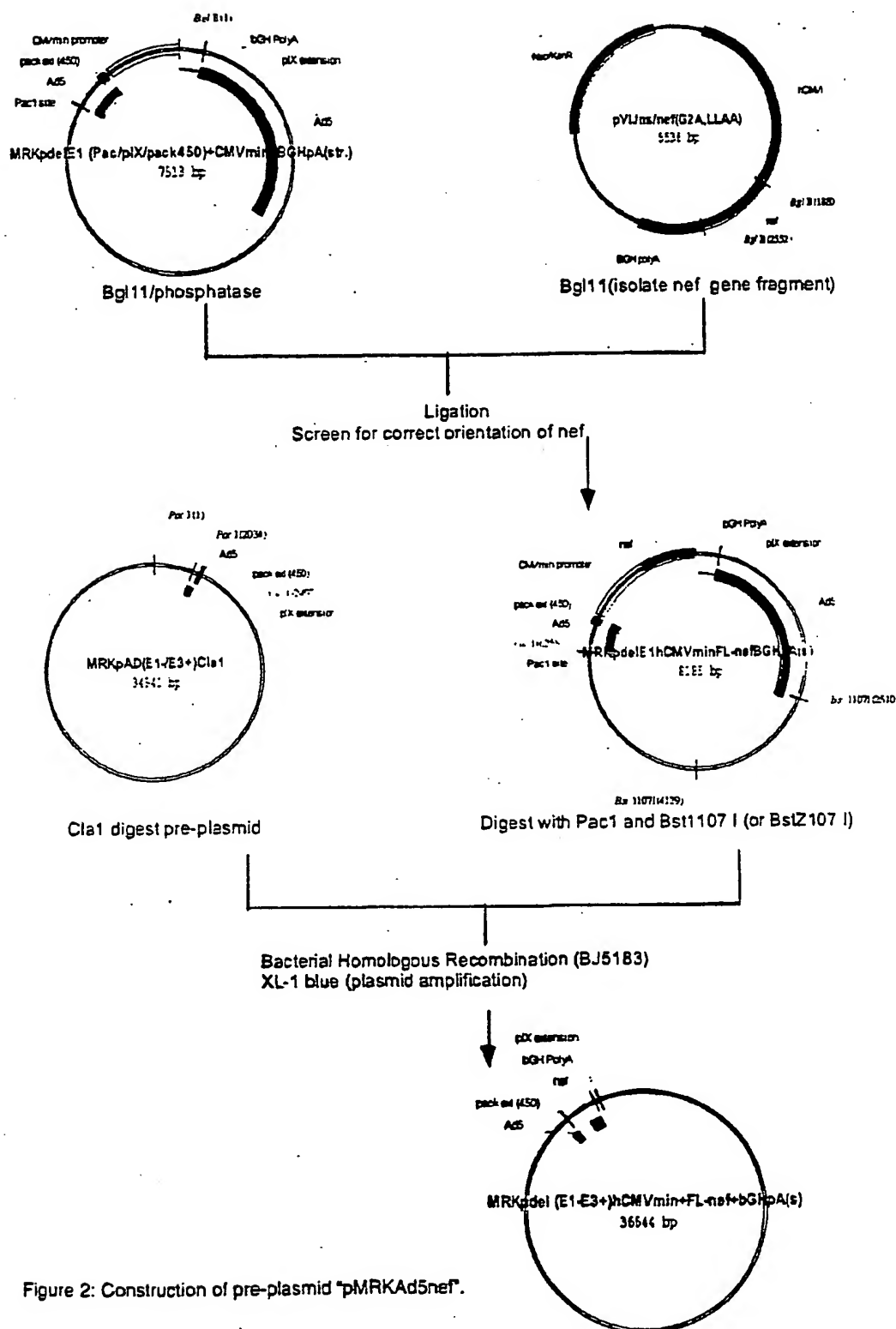
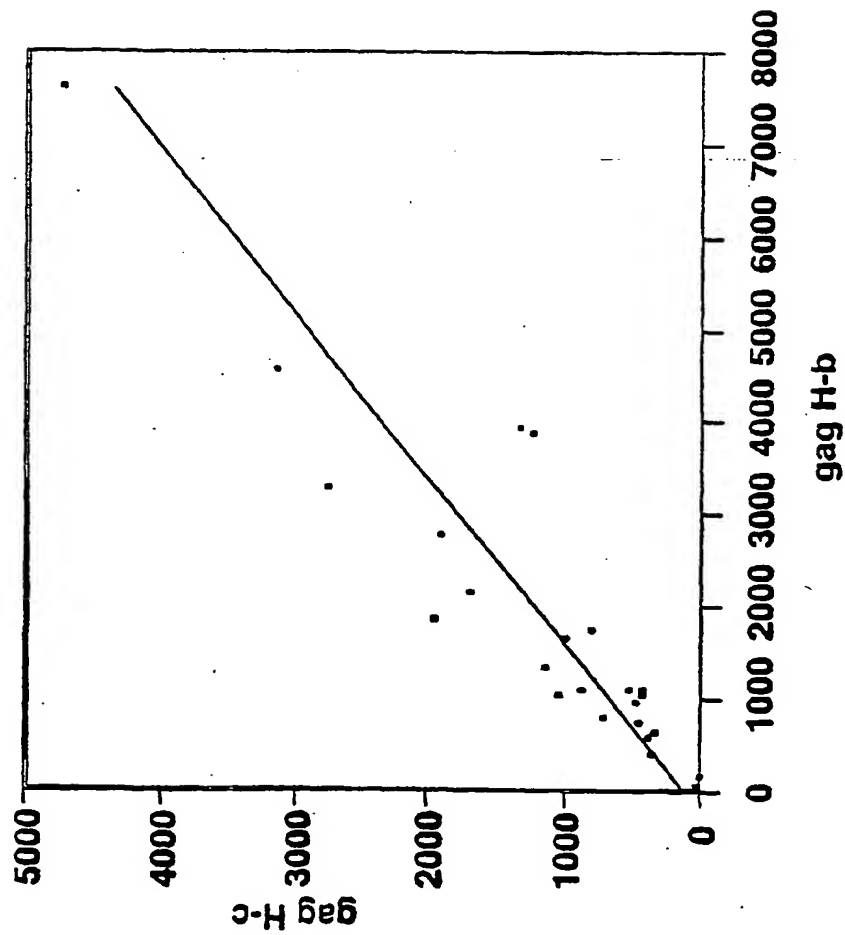


Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



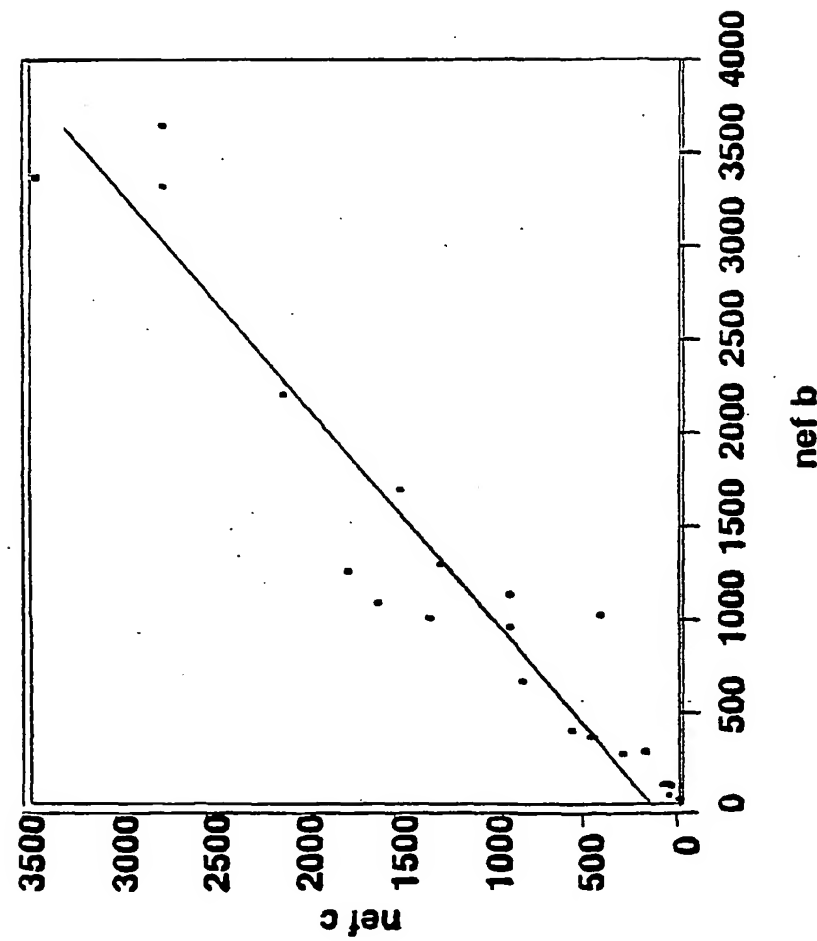
Linear Fit

$$\text{gag H-c} = 111.603 + 0.55866 \text{ gag H-b}$$

Summary of Fit

RSquare	0.816775
RSquare Adj	0.80914
Root Mean Square Error	474.9639
Mean of Response	1158.115
Observations (or Sum Wgts)	26

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



nef c = 131.132 + 0.8646 nef b

Summary of Fit	
RSquare	0.91685
RSquare Adj	0.91289
Root Mean Square Error	289.7718
Mean of Response	1096.435
Observations (or Sum Wgts)	23

FIGURE 25

MRKAd5pol MER1062
(MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAACTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAAC TG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTGTCTA
   TCACCTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGCGCAAG GCCCAGTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT TACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGGCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACGGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTAATTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

```

Figure 26A

901 TCGCTATTAC C GGTGATG CGGTTTGGC AGTACATCAA TGGGCG EA
 AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT
 951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
 ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT
 1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
 ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT
 1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
 TGTGAGGCG GGGTAACGTC GTTTACCCGC CATCCGCACA TGCCACCCTC
 1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
 CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC
 1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
 GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG
 1201 TCCGCGGCGG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
 AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA
 1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC
 CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG
 1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG
 ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC
 1351 GAGAAGATCA AGGCCCTGGT GGAATCTGC ACTGAGATGG AGAAGGAGGG
 CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCTCC
 1401 CAAATCTCC AAGATTGGCC CCGAGAACC CTACAACACC CCTGTGTTTG
 GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC
 1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG
 GGTAGTTCTT CTTCCTGAGG TGTTTCACCT CCTTCGACCA CCTGAAGTCC
 1501 GAGTGAAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC
 CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG
 1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG
 GGTGGGGCGA CCGGACTTCT TCTTCTTCAG ACACTGACAC GACCGACACC
 1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT
 CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTTATGTGA
 1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA
 CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT
 1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT
 CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA
 1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
 GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA
 1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT
 CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 24B

1851 TGGGCAGCAC A CCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTG T
 ACCCGTCGTG TCCTGGTTCT AACTCCTCGA CTCCGTCGTG GACGACTCCA
 1901 GGGGCCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG
 CCCCGGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC
 1951 TGGATGGGCT ATGAGCTGCA CCCCGACAAG TGGACTGTGC AGCCCATTTGT
 ACCTACCCGA TACTCGACGT GGGGCTGTTT ACCTGACACG TCGGGTAACA
 2001 GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG
 CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC
 2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
 CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC
 2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT
 GACACGTTCC ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA
 2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG
 CTGACTCCTC CGACTCGACC TCGACCGACT CTGTCCCTC TAGGACTTCC
 2201 AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG
 TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC
 2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC
 TAGGTCTTCG TCCCGGTCCC GGTCACCTGG ATGGTTTAGA TGCTCCTCGG
 2301 CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCCACA
 GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCC GGGTGT
 2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
 GGTACTACA CTTCGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC
 2401 TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA
 AGGTAACACT AGACCCCGTT CTGGGGGTTT AAGTTCGACG GGTAGGTCTT
 2451 GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC
 CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG
 2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG
 GACTCACCTT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC
 2551 CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC
 GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGGAAGATAC ACCGACCCCG
 2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG
 ACGGTTGTCC CTCTGGTTCC ACCCGTTCCG ACCGATACAC TGGTTGTCCC
 2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
 CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTCTGACGG
 2701 CTCAGGCCA TCTACCTGGC CTTCCAGGAC TCTGGCCTGG AGGTGAACAT
 GAGGTCCGGT AGATGGACCG GGAGGTCTCG AGACCGGACC TCCACTTGTA
 2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC
 ACACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

Figure 26C

2801 AGTCTGAGTC TCTGGTG AACCAGATCA TTGAGCAGCT GATCAA G
 TCAGACTCAG ACTCGACCAC TTGGTCTAGT AACTCGTCGA CTAGTTCTTC
 2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA
 CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCTCGT AACCCCGTT
 2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC
 ACTCGTCCAC CTGTTGACAC ACAGACGACC GTAGTCCTTC CACGACAAGG
 2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
 ACCTACCCTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG
 3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA
 ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT
 3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG
 CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC
 3101 GGCAGGTGGA CTGCTCCCTT GGCATCTGGC AGCTGGCCTG CACCCACCTG
 CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC
 3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA
 CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAAGT
 3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC
 CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG
 3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
 ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG
 3301 TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGTGCTG GGGCTGGCAT
 AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA
 3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCACTCCAG GGGGTGGTGG
 GTTCGTCTTC AAACCGTAGG GGATGTTGGG GGTGAGGGTC CCCCACCACC
 3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG
 GGAGGTACTT GTTCTCTGAC TTCTTCTAGT AACCCTCCA CTCCCTGGTC
 3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCT
 CGACTCGTGG ACTTCTGTG ACACGTCTAC CGACACAAGT AGGTGTTGAA
 3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG
 GTTCTCCTTC CCCCCGTAGC CCCCAGTAG GCGACCCCTC TCCTAACACC
 3551 ACATCATGTC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
 TGTAAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG
 3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG
 TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCTT TGGGGGACAC
 3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC
 CTTCCCGGGA CGGTTGACG ACACCTTCCC CCTCCCCCGA CACCACTAGG
 3701 AGGACAACCTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC
 TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

Figure 26 D

3751 AGGGACTATG CAGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCACA
 TCCCTGATAC CATTCTCTTA CCGACCCCTA CTGACACACC GGAGGTCTGT
 3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC
 CCTACTCCTG ATTCGGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCC
 3851 CATCTGTGTG TTGCCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC
 GTAGACAACA AACGGGGAGG GGGCACGGAA GGAAC TGGGA CCTTCCACGG
 3901 ACTCCCACTG TCCTTTCTTA ATAAAATGAG GAAATTGCAT CGCATTGTCT
 TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA
 3951 GAGTAGGTGT CATTCATATC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
 CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTCC
 4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
 CCTCTCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA
 4051 ATGGCCGATC GCGCGCCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG
 TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCCAC
 4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC
 CCTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG
 4151 AGCAGCCGCC GCCGCCATGA GCACCAATC GTTGATGGA AGCATTGTGA
 TCGTCGGCGG CCGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT
 4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT
 CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA
 4251 GTGATGGGCT CCAGCATTGA TGTGCGCCCC GTCTGCCCCG CAAACTCTAC
 CACTACCCGA GGTCTGTAAT ACCAGCGGGG CAGGACGGGC GTTTGAGATG
 4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT
 ATGGAACCTG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCCGA
 4351 CCGCGCCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC
 GCGCGCGGCG AAGTCGGCGA CGTCGCTGGC GGGCGCCCTA ACACTGACTG
 4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC
 AAACGAAAGG ACTCGGGCGA ACGTTTGTC ACGTGAAGGG CAAGTAGGCG
 4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC
 GCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG
 4501 GGGAACTTAA TGTGCTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGT
 CCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTCTGTCOA
 4551 TCTGCCCTGA AGGCTTCTC CCTCCCAAT GCGGTTTAAA ACATAAATAA
 AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT
 4601 AAAACCAGAC TCTGTTTGGG TTTGGATCAA GCAAGTGTCT TGCTGTCTTT
 TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA
 4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTGC
 TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCTC CAGAGCCAGC

Figure 26E

4701 TTGAGGGTCC TGTGSTATTTT TTCCAGGACG TGGTAAAGGT-GACTCTGAT
 AACTCCCAGG AATAAAAA AAGGTCTCTG ACCATTTCCTA CTGAGA A
 4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT
 CAAGTCTATG TACCCGTATT CGGCGAGAGA CCCCACCTCC ATCGTGGTGA
 4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG
 CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC
 4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC
 CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG
 4901 CAGGGGCAGG CCCTTGGTGT AAGTGTITAC AAAGCGGTTA AGCTGGGATG
 GTCCCGTCC GGAACACACA TTCACAAATG TTTCGCCAAT TCGACCCTAC
 4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG
 CCACGTATGC ACCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC
 5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC
 CGATACAAGG GTCGGTATAG GGAGGCCCCT AAGTACAACA CGTCTTGGTG
 5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCTGT AGCTTAGAAG
 GTCGTGTAC ATAGGCCACG TGAACCTTT AAACAGTACA TCGAATCTTC
 5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC
 CTTTACGCAC CTTCTTGAAC CTCTGCGGGA ACACGAGAGG TTCTAAAAGG
 5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC
 TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCGCC GCCGGACCCG
 5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT
 CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA
 5251 CGTCATAGCC CATTTTTACA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT
 GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCACGG TCTGACGCCA
 5301 ATAATGGTTC CATCCGCCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT
 TATTACCAAG GTAGGCCGGG TCCCCGATC AATGGGAGTG TCTAAACGTA
 5351 TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TGCGGGGCGA
 AAGGGTGCGA AACTCAAGTC TACCCCCCTA GTACAGATGG ACGCCCCGCT
 5401 TGAAGAAAAC GGTTCGCGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG
 ACTTCTPTTG CCAAAGGCC CATCCCTCT AGTCGACCCT TCTTTCGTCC
 5451 TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCCG AAATCACACC
 AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG
 5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC
 ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG
 5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC
 ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG
 5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTCTTGG
 GACTGGTTTA GCGGCTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

Figure 26F

5651 CAAGGAAGCA AATTTTTCA ACGGTTTGAG ACCGTCCGCC GTAGGCATC
 GTTCCTTCGT TTCAAAAAGT TGCCAAACTC TGGCAGGCGG CATCCGTACG
 5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC
 AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG
 5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG
 ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC
 5801 CGGCTTTTCG TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT
 GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA
 5851 CATGCTCTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTACGG
 GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC
 5901 TGAAGGGGTG CGCTCCGGGC TCGCGCTGG CCAGGGTGCG CTTGAGGCTG
 ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC
 5951 GTCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG
 CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC
 6001 GTAGCATTTC ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT
 CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACC GGGA
 6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA
 ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT
 6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA
 GAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCCTCAT
 6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG
 CCGTAGGCGC GCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC
 6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTCCTCCC ATGCTTTTTC
 ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC
 6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CCGTGTCCAC GCTCGGTGAC
 TACGCAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG
 6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCTCGA
 CTTTTCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT
 6351 GCGGTGTTC GCGGTCTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA
 CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT
 6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG
 TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTACACC TCCCCATCGC
 6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTA AGACACATGT
 CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACT TCTGTGTACA
 6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG
 GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC
 6551 TGACCGGGTG TTCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCGTTC
 ACTGGCCAC AAGGACTTCC CCCCATATT TTCCCCACC CCCGCGCAAG

Figure 266

6601 GTCTCACTC TCTTCCGCAT CGCTGTCTGC GAGGGCCAGT TCTGTGCTTG
 CAGGAGTGAG AGGCGTA GCGACAGAAG CTCCCGGTCTG ACAACGAC
 6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCACTT
 TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTG TAACAGTCAA
 6701 TCCAAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCCTT
 AGGTTTTCGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA
 6751 GAGGGTGGCC GCATCCATCT GGTGAGAAAA GACAATCTTT TTGTTGTCAA
 CTCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAACAGTT
 6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTGGCGGATG
 CGAACCCCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC
 6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCCGGAT
 CTCGCGTCCC AAACCAAAAA CAGCGCTAGC GCGCGAGGA ACCGGCGCTA
 6901 GTTAGCTGC ACGTATTGCG GCGCAACGCA CCGCCATTG GGAAGACGG
 CAAATCGACG TGCATAAGCG CGGTTGCGT GCGGTAAGC CCTTCTGCC
 6951 TGGTGCCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG
 ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCG CAAACGCTCC
 7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT
 CACTGTTCCA GTTGCACCA CCGATGGAGA GCGCATCCG CGAGCAACCA
 7051 CCAGCAGAGG CGGCCGCCCT TGCAGGAGCA GAATGGCGGT AGGGGGTCTA
 GGTGCTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT
 7101 GCTGCGTCTC GTCCGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCGC
 CGACGCGAG CAGGCCCCCC AGACGCGAGT GCCATTTCTG GGGCCGCTCG
 7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CCTTGCAAGT CTAGCGCCTG
 TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC
 7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC
 GACGTACGC GCCCGCCGTT CCGCGCGGAG CATACCCAAC TCACCCCTG
 7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTG
 GGGTACCGTA CCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC
 7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT
 ATTTGCATCT CCCCAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA
 7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTG TCGAGGGGAG
 AGGTGGCGCC TACGACCGCG CGTGCAATTAG CATATCAAGC ACGCTCCCTC
 7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG
 GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC
 7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG
 TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC
 7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCAGGAAG
 CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

7551 AGGCGTAGGA GCGCAGC TTGTTGACCA GCTCGGCGGT GACCTG
 TCCGCATCCT CAGCGCGTCG AACAACTGGT CGAGCCGCCA CTGGACGTGC
 7601 TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG
 AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC
 7651 TCCCTTTTTT TTCCACAGCT CGCGGTGAG GACAACTCT TCGCGGTCTT
 AGGGAAAAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA
 7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
 AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA
 7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
 TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG
 7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCGG GAGCGAGGTG TGGGTGAGCG
 CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC
 7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
 GTTTCACAG GGA CTGGTAC TGAACTCCA TGACCATAAA CTTCAGTCAC
 7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTGGGA
 AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT
 7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG
 TGCGCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC
 8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGTTCCCG CACCTCGGAA
 GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT
 8051 CGGTGTGTTAA TTACCTGGGC GCGGAGCAGC ATCTCGTCAA AGCCGTGAT
 GCCAACAAAT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA
 8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
 CAACACCGGG TGTTACATTT CAAGGTTCCT CCGCCCTAC GGGAACTACC
 8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
 TTCCGTAAA AAATCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG
 8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
 GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCAACC TTCGCTGCTT
 8251 TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
 ACTCGAGGTG TCCAGTGCCC GGTAATCGTA AACGTCCACC AGCGCTTTC
 8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG
 AGGATTTGAC CGCTGCATAC CCGTAAAAAA GACCCCACTA CGTCATCTTC
 8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTTCG CGGCTAGGTC
 CATTGCCCCA GAACAAGGGT CGCCAGGGTA GGTTCGAAGC GCCGATCCAG
 8401 TCGCGCGGCA GTCAC TAGAG GTCATCTCTC GCCGAAC TTC ATGACCAGCA
 AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CCGCTTGAAG TACTGGTCGT
 8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT
 ACTTCCCGTG CTCGACGAAG GGTTCGCGG GGTAGGTTCA TATCCAGAGA

Figure 26I

8501 ACATCGTAGG TAAAGAG ACGCTCGGTG CGAGGATGCG AGCCGA
 TGTAGCATCC ACTGTTTCTC TCGAGCCAC GTCCTACGC TCGGCTAGCC
 8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT
 CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA
 8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTA
 CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT
 8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG
 TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC
 8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCTT
 CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCTTA AACTCGGGGA
 8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTGCGCTGC TTGTCCTTGA
 GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT
 8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG
 GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC
 8851 CGAGCCCAA GTCCAGATGT CCGCGCGCGG CGGTGCGAGC TTGATGACAA
 GCTCGGGTTT CAGGTCTACA GCGCGCGCGC GCCAGCCTCG AACTACTGTT
 8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGCGCTCAGG
 GTAGCGCGTC TACCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC
 8951 TCAGGCGGGA GCTCCTGCAG GTTACCTCG CATAGACGGG TCAGGGCGCG
 AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC
 9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT
 CCGATCTAGG TCCACTATGG ATTAAGGTC CCCGACCAAC CACCGCCGCA
 9051 CGATGGCTTG CAAGAGGCGG CATCCCCGCG GCGCGACTAC GGTACCGCGC
 GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG
 9101 GCGGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAGCGG
 CCGCCCGCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCG
 9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG
 ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GGCGGCCCTC
 9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG
 TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCCTCG ACCACGACGC
 9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GGCGGTTGAT CTCTGAATC
 GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG
 9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCG GTGAGCTTGA ACCTGAAAGA
 ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT
 9351 GAGTTCGACA GAATCAATTT CGGTGTCGTT GACGGCGGCC TGGCGCAAAA
 CTCAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCGG ACCGCGTTTT
 9401 TCTCTGCAC GTCTCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC
 AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

9451 TGCTCGATCT C CTCTCTG GAGATCTCCG CGTCCGGCTC GCTCCA T
 ACGAGCTAGA GAAGGAGGAC CTCTAGAGGC GCAGGCCGAG CGAGGTGCCA
 9501 GGCGGCGAGG TCGTTGGAAA TCGGGGCCAT GAGCTGCGAG AAGGCCGTTGA
 CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT
 9551 GGCTTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG
 CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC
 9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA
 GCGCGCGCGT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT
 9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG
 CTGCCGCTATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC
 9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTTCG
 ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC
 9751 TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC
 AACTATAGGG GGTTCGGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG
 9801 GGCGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT
 CCGCTTCAAC TTTTGTACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA
 9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG
 GGTCTTCTGC TACTCGAGC CGCTGTACA GCGCGTGGAG CGCGAGTTTC
 9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC
 CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG
 9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC
 GGAAGAAGA AGAAGACCGC GCCCACCCCC TCCCCCTGT GCCGCCGCTG
 10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG
 CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC
 10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG
 GCTGCCCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCGCGTC
 10101 TTGGAAGACG CCGCCCCGTC TGTCCCGTT ATGGGTGGC GGGGGGCTGC
 AACCTTCTGC GCGGGGAGT ACAGGGCCAA TACCAACCG CCCCCGACG
 10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA
 GTACGCCGTC CTTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT
 10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA
 CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT
 10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAGGCTGA
 TTTGGAGAGC TCTTTCCGCA GATTGGTCAG TGTACGCGTT CCATCCGACT
 10301 GCACCGTGGC GGGCGGCGAC GGGCGGCGGT CGGGGTGTT TCTGGCGGAG
 CGTGGCACCG CCCGCCGTCG CCCGCCGCCA GCCCCAACAA AGACCGCCTC
 10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GCGGGATGGT
 CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10401 CGACAGAAGC AATGTCTCT TGGGTCCGGC CTGCTGAATG CGCAGGCTCT
 GCTGTCTTCG TTTTACAGGA ACCCAGGCCG GACGACTTAC GCGTCCCTCA
 10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GCGCAGGTC TTTGTAGTAG
 CCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC
 10501 TCTTGCAATG GCCTTTCTAC CGGCACCTCT TCTTCTCCTT CCTCTGTCC
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG
 10551 TGCATCTCTT GCATCTATCG CTGCGCGCGC GCGGAGTTT GCGCGTAGGT
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCTCAA CCGGCATCCA
 10601 GCGGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCCT CATCGGCTGA
 CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT
 10651 AGCAGGGCTA GGTCCGCGAC AACCGGCTCG GCTAATATGG CCTGTGTCAC
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC
 10751 CGCCCGTGTT GATGGTGTA GTCAGTTGG CCATAACGGA CCAGTTAAGC
 GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC
 10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GCGTGGTCC ATGACCATAG
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG
 GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGT CGCATCCAC
 10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA
 CGGCCCGAG GCCCGGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT
 11001 GATGTACCTG GACATCCAGG TGATGCCGGC GCGGGTGGTG GAGGCGCGCG
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC
 11051 GAAAGTCGCG GACGCGGTT CAGATGTTGC GCAGCGGCAA AAAGTGCTCC
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG
 11101 ATGGTCGGGA CGCTCTGGCC GGTCAAGGCG GCGCAATCGT TGACGCTCTA
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CCGCTTAGCA ACTGCGAGAT
 11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG
 CTGGCACGTT TTCTCTCTCG ACATTGCCCC GTGAGAAGGC ACCAGACCAC
 11201 GATAAAATTC CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA
 CTATTTAAGC GTTCCCATAG TACCGCTGCG TGGCCCCAAG CTCGGGGCAT
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGGT GTCGAACCCA
 AGGCCGGCAG GCGGCACTAG GTACGCCAAT GCGGGGCGCA CAGCTTGGGT
 11301 GGTGTGCGAC GTCAGACAAC GGGGAGTGC TCCTTTTGGC TTCTTCCAG
 CCACACGCTG CAGTCTGTTG CCCCCTCAG AGGAAAACCG AAGGAAGGTC

Figure 26L

11351 GCGCGGCGGC TGGCGCTA GCTTTTTTGG CCACTGGCCG CGCGCACT
 CGCGCCGCCG AGGACGCGAT CGAAAAACC GGTGACCGGC GCGCGTCCCA
 11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATT AAGTGGCTCGC TCCCTGTAGC
 TTCGCCAATC CGACCTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG
 11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCG GTTCGAGTCT
 GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA
 11501 CGGACCGGCC GGAATGCGGC GAACGGGGGT TTGCTTCCC GTCATGCAAG
 GCCTGGCCGG CCGACGCGC CTTGCCCCCA AACGGAGGGG CAGTACGTTT
 11551 ACCCGGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTGTGCTT
 TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA
 11601 TTCCAGATG CATCCGGTGC TCGGCGAGAT GCGCCCCCT CCTCAGCAGC
 AAGGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTGC
 11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCCT CCCTCCTCCT
 CCGTTCTCGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA
 11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA
 TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT
 11751 TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG
 AATGCTTGGG GCGCGCGCGG CCCGGGCGCT GATGGACCTG AACCTCCTCC
 11801 GCGAGGGCCT GCGCGGCTA GGAGCGCCCT CTCTGAGCG GCACCCCAAGG
 CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTC
 11851 GTGCAGCTGA AGCGTGATAC GCGTGAGCG TACGTGCCGC GGCAGAACCT
 CACGTCGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGA
 11901 GTTTCGCGAC CCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT
 CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA
 11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCA GCGGTTGCTG
 AGGTGCGTCC CCGCTCGAC GCCGTACCG ACTTAGCGCT CGCCAACGAC
 12001 CCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG
 GCGTCTCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC
 12051 CGCACACGTG GCGGCCGCGG ACCTGGTAAC CGCATACGAG CAGACGGTGA
 GCGTGTGCAC CCGCGCGCGC TGGACCATG GCGTATGCTC GTCTGCCACT
 12101 ACCAGGAGAT TAACCTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT
 TGGTCTCTA ATTGAAAGTT TTTTCGAAAT TGTGGTGCA CGCATGCGAA
 12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT
 CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA
 12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCGGCTCATG GCGCAGCTGT
 TTCGCGCGAC CTCGTTTTGG GTTTATCGTT CGGCGAGTAC CCGCTCGACA
 12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTAG GGATGCGCTG
 AGGAATATCA CGTCGTGTCG TCCTGTGTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12301 CTAAACATAG T G C C C G A G G G C C G C T G G C T G C T C G A T T T G A T A A T
 G A T T T G T A T C A T C T C G G G C T C C C G G C G A C C G A C G A G C T A A A C T A T T T G T A

12351 C C T G C A G A G C A T A G T G G T G C A G G A G C G C A G C T T G A G C C T G G C T G A C A A G G
 G G A C G T C T C G T A T C A C C A C G T C C T C G C G T C G A A C T C G G A C C G A C T G T T C C

12401 T G G C C G C C A T C A A C T A T T C C A T G C T T A G C C T G G G C A A G T T T T A C G C C C G C
 A C C G C G G T A G T T G A T A A G T A C G A A T C G G A C C C G T T C A A A A T G C G G G C G

12451 A A G A T A T A C C A T A C C C C T T A C G T T C C C A T A G A C A A G G A G T A A A G A T C G A
 T T C T A T A T G G T A T G G G A A T G C A A G G G T A T C T G T T C C T C C A T T T C T A G C T

12501 G G G G T T C T A C A T G C G C A T G G C G C T G A A G G T G C T T A C C T T G A G C G A C G A C C
 C C C C A A G A T G T A C G C G T A C C G C G A C T T C C A C G A A T G A A C T C G C T G C T G G

12551 T G G G C G T T T A T C G C A A C G A G C G C A T C C A C A A G G C C G T G A G C G T G A G C G T G A G C C G G
 A C C C G C A A A T A G C G T T G C T C G C G T A G G T G T T C C G G C A C T C G C A C T C G G C C

12601 C G G C G C G A G C T C A G C G A C C G C G A G C T G A T G C A C A G C C T G C A A A G G G C C C T
 G C C G C G C T C G A G T C G C T G G C G C T C G A C T A C G T G T C G G A C G T T T C C C G G G A

12651 G G C T G G C A C G G G C A G C G C G A G G G C A T A G A G A G G C C G A G T C C T A C T T T G A C G C G G
 C C G A C C G T G C C C G T C G C C G C T A T C T C T C C G G C T C A G G A T G A A A C T G C G C C

12701 G C G C T G A C C T G C G C T G G G C C C A A G C C G A C G C G C C C T G G A G C A G C T G G G
 C G C G A C T G G A C G C G A C C C G G G G T T C G G C T G C G C G G A C C T C C G T C G A C C C

12751 G C C G G A C C T G G C T G G C G G T G G C A C C C G C G C G C T G G C A A C G T C G G C G G
 C G G C C T G G A C C G A C C C C A C C G T G G G C G C G C G C A C C G T T G C A G C C G C C

12801 C G T G G A G G A A T A T G A C G A G G A C G A T G A G T A C G A G C C A G A G A C G G C G A G T
 G C A C C T C C T T A T A C T G C T C C T G C T A C T C A T G C T C G G T C T C T G C C C G C T A

12851 A C T A A G C G G T G A T G T T T C T G A T C A G A T G A T G C A A G A C G C A A C G G A C C C G G
 T G A T T C G C C A C T A C A A G A C T A G T C T A C T A C G T T C T G C G T T G C C T G G G C C

12901 C G G T G C G G G C G G C G C T G C A G A G C A G C C G T C C G G C C T T A A C T C C A C G G A C
 G C C A C G C C C G C C G C G A C G T C C G G T C G G C A G G C C G G A A T T G A G G T G C C T G

12951 G A C T G G C G C C A G G T C A T G G A C C G A T C A T G T C G C T G A C T G C G C A A T C C
 C T G A C C G C G G T C C A G T A C C T G G C G T A G T A C A G C G A C T G A C G C G C G T T A G G

13001 T G A C G C G T T C C G C A G C A G C C G A G G C C A A C C G G C T C T C C G C A A T T C T G G
 A C T G C G C A A G G C C G T C G T C G C G C G T C C G G T T G G C C G A G A G G C G T T A A G A C C

13051 A A G C G G T G G T C C C G G C G C G C G C A A A C C C C A C G C A C G A G A A G G T G C T G G G C
 T T C G C C A C C A G G C C G C G C G T T T G G G G T G C G T G C T C T T C C A C G A C C G C

13101 A T C G T A A A C G C G C T G G C C G A A A C A G G G C C A T C C G G C C C G A C G A G G C C G G
 T A G C A T T T G C G C A C C G G C T T T G T C C C G G T A G G C C G G G C T G C T C C G G C C

13151 C C T G G T C T A C G A C C G C T G C T T C A G C G C G T G G C T C G T T A C A A C A G C G G C A
 G G A C C A G A T G C T G C G C A C G A A G T C G C G C A C C G A G C A A T G T T G T C G C C G T

13201 A C G T G C A G A C C A A C C T G G A C C G G C T G G T G G G G A T G T G C G C G A G G C C G T G
 T G C A C G T C T G G T T G A C C T G C C G A C C A C C T A C A C G C G C T C C G G C A C

Figure 26 N

13251 GCGCAGCGTG ACGCGCA GCAGCAGGCG AACCTGGGCT CCATGGGTC
 CGCGTCGCAC TCGCGCGCGT CGTCGTCCCG TTGGACCCGA GGTACCAACG
 13301 ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG
 TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGACAGGC GCCCTGTCC
 13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA
 TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT
 13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG
 GCGCTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC
 13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAAGTTGC
 ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTGAACG
 13501 AGGGGCTGTG GGGGGTGC GGCTCCACAG GCGACCGCGC GACCGTGTCT
 TCCCCGACAC CCCCCACGCC CGAGGCTGTC CGCTGGCGCG CTGGCACAGA
 13551 AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT
 TCGAACGACT GCGGGTTGAG CGCGACAAC GACGACGATT ATCGCGGGAA
 13601 CACGGACAGT GCGAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA
 GTGCCGTGCA CCGTCGCACA GGGCCTGTG TATGGATCCA GTGAACGACT
 13651 CACTGTACCG CGAGGCCATA GGTGAGGCGC ATGTGGACGA GCATACTTTC
 GTGACATGGC GCTCCGTAT CAGTCCGCG TACACCTGCT CGTATGAAAG
 13701 CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG
 GTCTCTAAT GTTCACAGTC GCGCGCGAC CCCGTCTCC TGTGCCCGTC
 13751 CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC
 GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG
 13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG
 GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC
 13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCAGCGT
 GTCGTCTGCG ACTCGGAATT GGAATACGCG CTGCCCCATT GCGGGTCGCA
 13901 GCGCGTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA
 CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT
 13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCACTG CGCGGCCGCC
 TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG
 14001 GTGAACCCCG AGTATTTAC CAATGCCATC TTGAACCCCG ACTGGCTACC
 CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG
 14051 GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCCGAG GGTAAACGATG
 CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC
 14101 GATTCTCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG
 CTAAGGAGAC CTGCTGTAT CTGCTGTGCG ACAAAGGGG CGTTGGCGTC
 14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA
 TGGGACGATC TCAACGTTGT CCGCTCGTC CGTCTCCGCC GCGACGCTT

Figure 260

14201 GGAAAGCTTC CCGAGGCCAA GCAGCTTGTC CGATCTAGGC GCTGCGTCC
CCTTTTCGAAG GCTCCGGTT CGTCGAACAG GCTAGATCCG CGACGC
14251 CGCGGTGAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC
GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG
14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GCGGAGGAGG AGTACCTAAA
TCGTGAGCGT GGTGGGCGGG GCGGACGAC CCGCTCCTCC TCATGGATTT
14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTT
GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GCGCGTAAAG
14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG
GGTTGTGCCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC
14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG
ATGCGCGTCC TCGTGTCCCT GCACGGTCCG GCGCGGGGCG GGTGGGCAGC
14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG
AGTTTCCGTG CTGGCAGTCG CCCAGACCA CACCTCCTG CTA CTGAGCC
14551 CAGACGACAG CAGCGTCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG
GTCTGTGTGTC GTGCGAGGAC CTAAACCCTC CTTACCGTT GGCAAAACGC
14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAATAA AAAAGCATGA
GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT
14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGTTTTCTT
ACGTTTTATT TTTTGTAGTG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA
14701 GTATTCCCCT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCTCCT
CATAAGGGGA ATCATACGCC GCGCGCCGCT ACATACTCCT TCCAGGAGGA
14751 CCCTCTTACG AGAGTGTGGT GAGCGCGGCG CCACTGGCGG CGGCGCTGGG
GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCAACGCC GCCGCGACCC
14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTGTGCTT CCGCGGTACC
AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG
14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC
ACGCCGATG GCCCCCTCT TGTGCTAGG CAATGAGACT CAACCGTGGG
14901 CTATTGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT
GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCTTACA
14951 GGCACTCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA
CCGTAGGGAC TTGATGGTCT TGCTGGTGTG GTTGAAAGAC TGGTGCCAGT
15001 TTCAAACAA TGA CTACAGC CCGGGGAGG CAAGCACACA GACCATCAAT
AAGTTTGTGTT ACTGATGTCG GGCCCCCTCC GTTCTGTGTG CTGGTAGTTA
15051 CTTGACGACC GGTGCACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC
GAATGCTGG CCAGCGTGAC CCCGCGCTG GACTTTTGGT AGGACGTATG
15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGCGC
GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATCCGCG

Figure 26 P

15151 GGGTGATGGT GCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTAA
 CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT
 15201 TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT
 ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA
 15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG
 CTGGTATCTG GAATACTTGT TCGCTAGCA CCTCGTGATG AACTTTCACC
 15301 GCAGACAGAA CGGGGTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC
 CGTCTGTCTT GCCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG
 15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG
 GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC
 15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT
 CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCTTA
 15451 GCGGGGTGGA CTTCACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC
 CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG
 15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA
 TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT
 15551 GGGTGGTAAC ATTCCCGCAC TGTGGATGT GGAAGCCTAC CAGGCGAGCT
 CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA
 15601 TGAAAGATGA CACCGAACAG GCGGGGGGTG GCGCAGGCGG CAGCAACAGC
 ACTTTCTACT GTGGCTTGTC CCGCCCCAC CGCGTCCGC GTCGTTGTCG
 15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA
 TCACCGTCGC CGCGCTTCT CTGAGGTTG CGCCGTCGGC GCCGTACGT
 15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA
 CGGCCACCTC CTGTACTTGC TAGTACGTA AGCGCCGCTG TGGAACGGT
 15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCGG AAGCAGCGGC CGAAGCTGCC
 GTGCCCGACT CCTCTTCGCG GACTCCGGC TTCGTGCGCG GCTTCGACGG
 15801 GCCCCCGCTG CGCAACCCGA GGTGAGAAG CCTCAGAAGA AACCAGTGAT
 CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA
 15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA
 GTTTGGGGAC TGTCTCCTGT CGTTCTTTC GTCAATGTTG GATTATTCGT
 15901 ATGACAGCAC CTTCACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC
 TACTGTCGTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG
 15951 GCGCACCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA
 CCGCTGGGAG TCTGGCCTTA GCGAGTACC TGGGACGAAA CGTGAGGACT
 16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC
 GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG
 16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG
 TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 A

16101 GTGGGCGCCG AATGTTGCC CGTGCACTCC AAGAGCTTCT ACAACGTA CA
 CACCCGCGGC TCAACAACGG GCACGTGAGG TTCTCGAAGA TGTTCGCT
 16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT
 CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA
 16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC
 AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG
 16251 ATCACCACCG TCAGTGAAAA CGTTCCTGCT CTCACAGATC ACGGGACGCT
 TAGTGGTGCG AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA
 16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG
 TGGCGACCGG TTGTCGTAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC
 16351 CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG
 GGCTGCGGC GTGGACGGGG ATGCAAAATGT TCCGGGACCC GTATCAGAGC
 16401 CCGCGCGTCC TATCGAGCCG CACTTTTTEA GCAAGCATGT CCATCCTTAT
 GCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCGTACA GGTAGGAATA
 16451 ATCGCCACGC AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT
 TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TC GTTCTACA
 16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGC CGGG
 AACCGCCCCG GTTCTTCGCG AGGCTGGTTG TGGGTACGCG GCACGCGCCC
 16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC
 GTGATGGCGC GCGGGACCCC GCGCGTGT TT GCGCCGGCGT GACCCGCGTG
 16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA
 GTGGCAGCTA CTGCGGTAGC TCGGCCACCA CCTCCTCCGC GCGTTGATGT
 16651 CGCCACGCC GCCACCAAGT TCCACAGTGG ACGCGGCCAT TCAGACCGTG
 GCGGGTGCGG CGGTGGTCAC AGGTGTCAAC TCGCCCGGTA AGTCTGGCAC
 16701 GTGCGCGGAG CCCGGCGCTA TGCTAAATG AAGAGACGGC GGAGGCGCGT
 CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA
 16751 AGCAGTTCG CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGCGCG
 TCGTGCAGCG GTGCGGGCGG CTGGGCCGTG ACGGCGGGTT GCGCGCGCGC
 16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGC GGCCATGCGG
 GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC
 16851 GCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG
 CGGCGAGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GGTCCAGGT
 16901 GCGACGAGCG GCCGCCGAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG
 CGCTGCTCGC CGGCGGCGTC GTGCGCGCCG GTAATCACGA TACTGAGTCC
 16951 GTCGCAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC
 CAGCGTCCCC GTTGACATA ACCCAGCGCG TGAGCCAATC GCCGGACGCG
 17001 GTGCCCCTGC GCACCCGCCC CCCGCGCAAC TAGATTGCAA GAAAAAATA
 CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT

Figure 26 R

17051 CTTAGACTCG TTTGTTGTA TGTATCCAGC GCGCGCGGCG CCGAACCTG
 GAATCTGAGC ATGACAACAT ACATAGGTG CCGCCGCCGC GCGTTGCTTC

17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG
 GATACAGGTT CCGCTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCGCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA
 CTCCTAGATAC CCGGGGGCTT CTTCTTCTC GTCCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG
 CGATTTCCGC CAGTTTCTT TTTCTTCTT ACTACTACTA CTTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG
 TGCTCCACCT TGACGACGTG CGATGCGCGG GGTCCGCTGC CCATGTCACC

17301 AAAGGTTCGAC GCGTAAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT
 TTTCCAGCTG CGCATTTTGC ACAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCGGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG
 ATGCGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG
 ACATGCCGCT GCTCCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA
 AAACGGATGC CTTTCGCCGT ATTCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC
 CCCGTGGGT TGTGGATCGG ATTTCCGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT
 GCGCGAAGC TGGCAGGCTT CTTTTCGCGC CGGATTTGCG GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA
 CTGAACCGTG GGTGGCACGT GACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAAATGA CCGTGAACC TGGGCTGGAG CCCGAGGTCC
 TCTACAGAAC CTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG

17701 GCGTGCGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG
 CGCACGCCG TTAGTTCGTC CACCGCGGCC CTGACCGCA CGTCTGGCAC

17751 GACGTTTCTA TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA
 CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GCGGCTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCGTTGTC CTCAGCGGTG GCGGATGCCG
 CCCGTACCTC TGTGTTTGCA GGGGCCAAGC GAGTCGCCAC CGCCTACGGC

17851 CCGTGCAGGC GGTGCTGCG GCCGCGTCCA AGACCTCTAC GGAGGTGCAA
 GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GATGTTTTCG CGTTTCAGCC CCGCGCGGCC CGCGCCGTTT
 TGCCTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCGCGG GCGCGGCAAG

17951 GAGGAAGTAC GCGCGGCCA GCGCGCTACT GCGCGAATAT GCCCTACATC
 CTCCTTCATG CCGCGGCGGT CCGCGGATGA CCGGCTTATA GCGGATGTAG

Figure 265

18001 CTTCCATTGC GCTACCCCC GGCTATCGTG GCTACACCTALCGGCCCCGGA
 GAAGGTAACG CATTGGGGG CCGATAGCAC CGATGTGGAT GGCGGGCT
 18051 AGACGAGCAA CTACCCGACG CCGAACCCACC ACTGGAACCC GCCGCCGCCG
 TCTGCTCGTT GATGGGCTGC GGCTTGCTGG TGACCTTGGG CGGCGGGCGG
 18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC
 AGCGGCAGCG GTCGGGCAGC ACCGGGGCTA AAGGCACGCG TCCCACCGAG
 18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCAGC
 CGCTTCTCC GTCCCTGGGAC CACGACGGT GTGCGCGCAT GTTGGGGTGC
 18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCTCACCTG
 TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC
 18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA
 GCGCGGAGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT
 18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TGCGCACCAC
 CCCCCTACCG GCCGGTGCCG GACTGCCCGC CGTACGCAGC ACCTGCTGGT
 18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CCGGGCGGTA TCCTGCCCTT
 GCGCGCGCGC GCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA
 18401 CTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT
 GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA
 18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG
 GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC
 18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCTGTAACT
 TTTTATGTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG
 18551 TATTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC
 ATAAACATC TTACCTTCTG TAGTTGAAC GCAGAGACCG GGGCGCTGTG
 18601 GGCTCGCGCC CGTTCATGGG AAAGTGGCAA GATATCGGCA CCAGCAATAT
 CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTCGTTATA
 18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT
 CTCGCCACCG CGGAAGTCGA CCCCAGCGA CACCTCGCCG TAATTTTAA
 18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGGA AGGCCTGGAA CAGCAGCACA
 AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTGCTGT
 18751 GGCCAGATGC TGAGGATAA GTTGAAAGAG CAAAATTTCC AACAAAAGGT
 CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTCCA
 18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGTGGAC CTGGCCAACC
 CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTGG
 18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCTTCCGTA
 TCGGTCACGT TTTATTCTAA TTGTCATTG AACTAGGGGC GGGAGGGCAT
 18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GGCGTGGCGA
 CTCTCGGAG GTGGCCGGCA CCTCTGTAC AGAGGTCTCC CCGCACCGCT

Figure 26T

18951 AAAGCGTCCG CCGGACA GGAAGAAAC TCTGGTGACG CAAATAGG
 TTTCGCAGGC GCGGGCTGT CCCTCTTTG AGACCACTGC GTTTATCSC
 19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT
 TCGGAGGGAG CATGCTCCTC CGTGATTTG TTCCGGACGG GTGGTGGGCA
 19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC
 GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG
 19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG
 CGACCTGGAC GGAGGGGGG GGCTGTGGGT CGTCTTTGGA CACGACGGTC
 19151 GCCCGACCGC CGTTGTGTGA ACCCGTCTTA GCCGCGCGTC CCTGCGCCGC
 CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACCGGGCG
 19201 GCCGCCAGCG GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA
 CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT
 19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC
 TTCTGTGTGAC TTGTCTGAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG
 19301 GACGATGCTT CTGATAGCTA ACGTGTGTA TGTGTGTCAT GTATGCGTCC
 CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG
 19351 ATGTCGCGCG CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA
 TACAGCGGCG GTCTCCTCGA CGACTCGGCG CGCGCGGGG GAAAGGTTCT
 19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC
 ACCGATGGGG AAGCTACTAC GCGGTCACCA GAATGTACGT GTAGAGCCCG
 19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCCGCG
 GTCTTCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG
 19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG
 GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC
 19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG
 GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAAA CTGCGACGCC
 19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT
 AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA
 19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT
 GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA
 19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT
 AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA
 19751 GGCACGCTT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA
 CCGTGACGGA TGTTCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT
 19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG
 TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC
 19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAACTCAC
 TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 u

19901 GTATTGGGC A GCCTTA TTCTGGTATA AATATTACAA AGGAGC T
 CATAAACCCG TCCGCGGAAT AAGACCATAT TTATAATGTT TCCTCCATA
 19951 TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTC
 AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG
 20001 AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT
 TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTTG TCTTTAATTA
 20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA
 GTACGTCGAC CCTCTCAGGA TTTTTCCTGA TGGGGTTACT TTGGTACAAT
 20101 CGGTTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG
 GCCAAGTATA CGTTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAAGAAC
 20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTC
 ATTTGCTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG
 20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT
 AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTCA
 20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT
 CCATAACATG TCACITCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA
 20301 CTTACATGCC CACTATTAAG GAAGGTAAGT CACGAGAACT AATGGGCCAA
 GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTTGA TTACCCGGTT
 20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT
 GTTAGATACG GGTTGTCCGG ATTAATGTAA CGAAATCCC TGTTAAATA
 20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC
 ACCAGATTAC ATAATGTGT CGTGCCCAT TATACCCACAA GACCGCCCGG
 20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG
 TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTCTGTC TTTGTGTCTC
 20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT
 GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA
 20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA
 AAGATACACC TTAGTCCGAC AACTGTGAT ACTAGGTCTA CAATCTTAAT
 20601 TTGAAAATCA TGGAACTGAA GATGAACTTC CAAATTACTG CTTTCCACTG
 AACTTTTAGT ACCTTGACTT CTAATTGAAG GTTTAATGAC GAAAGGTGAC
 20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAAACAGG
 CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC
 20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG
 AGTCCTTTTA CCTACCTTT TTCTACGATG TCTTAAAGT CTATTTTAC
 20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC
 TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG
 20801 CTGTGGAGAA ATTTCTGTGA CTCCAACATA GCGCTGTATT TGCCCGACAA
 GACACCTCTT TAAAGGACAT GAGGTTGTAT CCGACATAA ACGGGCTGTT

Figure 26 v

20851 GCTAAAGTAC AGCCTTCCA ACGTAAAAAT TTCTGATAAC CCAAATCTT
 CGATTTTCATG TGGGAAGGT TGCATTTTTA AAGACTATTG GGTTCGAA

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC
 TGCTGATGTA CTTGTTCGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC
 TAATTGGAAC CTCGTGCGAC CAGGGAAGTG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG
 TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCCTT
 CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA
 CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCGCA GAGCTCCCTA GGAAATGACC
 GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC
 ATTCCTCACT GCCTCGGTCTG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCCTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT
 TGAAGAAGG GTTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA
 ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC
 TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTTCACCGG GTATAGGTAG

21401 CCTTCCCGCA ACTGGGCGGC TTCCGCGGC TGGGCCTTCA CGCGCCTTAA
 GGGAGGGCGT TGACCCGCCG AAAGGCGCGC ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT
 CTGATTCTCT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC
 TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA
 AAATTCCTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG
 ACTGGCGGAC GAATGGGGGT TGCTCAAACCT TTAATTCGCG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAAAC TGACCAAAGA CTGGTTCCTG
 CCTCCCAAT GTTGCACCGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC
 CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA
 TCTCTCGATG TTCTGGCGT ACATGAGGAA GAAATCTTG AAGTTCGGGT

Figure 26 W

21801 TGAGCCGTCA GGTGGTGGAT GATACTAAAT ACAAGGACTA CCAACAGG
 ACTCGGCAGT CACACCTA CTATGATTTA TGTTCTGAT GGTGTAC

21851 GGCACTCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC
 CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA
 GTGGTACGCG CTTCTGTGCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT
 ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTC AAAGAAACGCTA

22001 CGCACCTTTT GCGGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC
 GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCACGCGC
 TGAGTGTCTG GACCCGGTTT TGAAGAGAT GCGGTTGAGG CGGGTGCCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCAC CTTCTTTAT
 ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTGTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACGCGG
 CAAAACAAAC TTCAGAACT GCACCAGGCA CACGTGCTCG GCGTGGCGC

22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA
 GCAGTAGCTT TGGCACATGG ACGCGTCCGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA
 GTTGATTTTC TTCGTTTCGT GTAGTTGTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATGTGCAAAG ATCTTGTTG TGGGCCATAT
 CACTCGTCTT TGACTTTCGG TAACAGTTT TAGAACCAAC ACCCGGTATA

22351 TTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTT CTCCACACAA
 AAAAACCCGT GGATACGTG CCGGAAAGGT CCGAAACAAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGCGGTAC
 CGAGCGGACG CGGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGATG

22451 ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT
 TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA
 CTCGGGAAAC CGAAAAGACT GGTGCTGAG TTCGTCCAAA TGGTCAAAC

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCGACCGCT
 CATGCTCAGT GAGGACCGCG CATCGCGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC
 CATATTGCGA CTTTTTCAGG TGGGTTTCG ATGTCCCCG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC
 CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCGG

22701 CCAAACCTCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC
 GGTGAGGG TACCTAGTGT TGGGGTGTA CTTGGAATAA TGGCCCCATG

Figure 26 x

22751 CCAACTCCAT GCTCAACAGT CCCCAGGTAC AGCCCACCGA GGGTGGGAC
 GGTGAGGTA CTTGTCA GGGGTCCATG TCGGGTGGGA CGCAGC
 22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCCGCAG
 GTCTTGTGCG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC
 22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTACAC TTGAAAAACA
 GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAACAGTG AACTTTTGT
 22901 TGTA AAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT
 ACATTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGAAAAATA
 22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT
 AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA
 23001 TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATCGCC ACTGGCAGGG
 AATTTTGTAGT TTCCCCAAGA CGCGCGTAG CGATACGCGG TGACCGTCCC
 23051 ACACGTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC
 TGTGCAACGC TATGACCACA AATCACGAGG TGAATTGAG TCCGTGTTGG
 23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC
 TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG
 23151 CAACGCGTTT AGCAGGTCGG GCGCGATAT CTGAAGTCG CAGTTGGGGC
 GTTGCGCAAA TCGTCCAGCC CGCGGTATA GAACTTCAGC GTCAACCCCG
 23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC
 GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG
 23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT
 TGATAGTCGC GGCCACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA
 23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT
 GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CTCAGTTGA
 23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC
 AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG
 23401 TCGCACCGTA GTGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG
 AGCGTGCGAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC
 23451 ATACAGCGCC TGCATAAAG CTTGATCTG CTAAAAGCC ACCTGAGCCT
 TATGTCGCGG ACGTATTTTC GGAAGTAGAC GAATTTTCGG TGGAAGTCGA
 23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AACTGATTG
 AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC
 23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTGAGAT
 CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA
 23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG
 GACGTGGTGT AAAGCCGGGG TGCCCAAGAA GTGCTAGAAC CGGAACGATC
 23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA
 TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26Y

23701 ATCACGTGCT C TATTTAT CATAATGCTT CCGTGTAGAC ACTTAATC
 TAGTGACAGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG
 23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT
 CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA
 23801 CGTGATGCTT GTAGSTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG
 GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC
 23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG
 TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC
 23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG
 GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC
 23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC
 GAAGGTGAAC CAGTCCGTCA TCAAACCTCA AGCGGAAATC TAGCAATAGG
 24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA
 TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT
 24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT
 GCGTCTGTGC TAGCCGTGTG AGTCGCCCCA GTAGTGGCAT TAAAGTGAAA
 24101 CCGCTTCGCT GGGCTCTTCC TCTTCTCTTT GCGTCCGCAT ACCACGCGCC
 GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGC GCGG
 24151 ACTGGGTCGT CTTCATTCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC
 TGACCCAGCA GAAGTAAGTC GCGGCGGTGA CACGCGAATG GAGGAAACGG
 24201 ATGCTTGATT AGCACCGGTG GGTGTGCTGAA ACCCACCATT TGTAGCGCCA
 TACGAATAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT
 24251 CATCTTCTCT TCTTCTCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG
 GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC
 24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC
 GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG
 24351 CAAATCCGCC GCCGAGGTG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA
 GTTTAGGCGG CGSCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT
 24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCTT CGGACTCGAT ACGCCGCCTC
 CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TCGGGCGGAG
 24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GCGGCGGACG GGGACGGGGA
 TAGGCGAAAA AACCCCGCG GGGCCCTCCG CGCCCGCTGC CCCTGCCCTT
 24501 CGACACGTCC TCCATGGTTG GGGGACGTG CGCCGCACCG CGTCCGCGCT
 GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA
 24551 CGGGGGTGGT TTCGCGTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC
 GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG
 24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC
 ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG

Figure 262

24651 CGCCCCCTCT GTCGCCA CCACCGCCTC CACCGATGCC GCAACGTC
 GCGGGGGAGA CTCAAGCGGT GGTGGCGGAG GTGGCTACGG CGGTTCGCGG
 24701 CTACCACCTT CCCCCTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT
 GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA
 24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT
 TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA
 24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG
 TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCCTCTC CGTTTGCTCC
 24851 AACAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA
 TTGTTCAAGC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT
 24901 GACGACGTGC TGTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA
 CTGCTGCACG ACAACTTCGT AGACGTGCGG GTCACGCGGT AATAGACGCT
 24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCTT CGCCATAGCG GATGTCAGCC
 GCGCAACGTT CTCGCTGCGC TACACGGGGA GCGGTATCGC CTACAGTCGG
 25001 TTGCCTACGA ACGCCACCTA TTCTCACC GCCTACCCCC CAAACGCCAA
 AACGGATGCT TCGGTGGAT AAGAGTGCGG CGCATGGGGG GTTTGCGGTT
 25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCGTATT
 CTTTTCGCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA
 25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACGCA
 ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAG GTTTTGACGT
 25151 AGATACCCCT ATCTGCGGT GCCAACGCA GCGGAGCGGA CAAGCAGCTG
 TCTATGGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTCGAC
 25201 GCCTTGCGGC AGGGCGCTGT CATACTGAT ATCGCCTCGC TCAACGAAGT
 CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA
 25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG
 CGGTTTPTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGPTTGC
 25301 CTCTGCAACA GGAAACAGC GAAAATGAAA GTCACCTCTG AGTGTTGGTG
 GAGACGTTGT CCTTTGTGCG CTTTACTTT CAGTGAGACC TCACAACCAC
 25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAC GCAGCATCGA
 CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTG CGTCGTAGCT
 25401 GGTCACCAC TTTGCCTACC CGGCACCTAA CCTACCCCC AAGGTCATGA
 CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGG TTCCAGTACT
 25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG
 CGTGTCAGTA CTCATCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC
 25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA
 CTACGTTTAA ACGTTCTTGT TTGTCTCTC CCGGATGGGC GTCAACCGCT
 25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG
 GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACCG CTGAACCTCC

Figure 26 AA

25601 AGCGACGCAA AATGATG GCCGCAGTGC TCGTTACCGT GGAGCTAG
TCGCTGCGTT TGATTACTAC CGGCGTCACG AGCAATGGCA CCTCGAACTC

25651 TGCATGCAGC GGTTCCTTTC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA
ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTGCGCT TCGATCTCCT

25701 AACATGTCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA
TTGTAACGTG ATGTGAAAAG CTGTCCCGAT GCATGCGGTC CGGACGTCT

25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC
AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAACACGTG

25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC
CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT
CGCGGCGCTG ATGCAGGCGC TGACGCAAAT GAATAAGAT ACGATGTGGA

25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC
CCGCTGCGCG GTACCCGCAA ACCGTCGTCA CGAACCTCCT CACGTTGGAG

25951 AAGGAGCTGC AGAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC
TTCCTCGACG TCTTTGACGA TTTCGTTTTG AACTTCCTGG ATACCTGCCG

26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GCGCGACATC ATTTTCCCG
GAAGTTGCTC GCGAGGCACC GCGCGTGGA CCGCCTGTAG TAAAAGGGG

26051 AACGCCTGCT TAAAACCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA
TTGCGGACGA ATTTTGGGAC GTTGTCCAG ACGGTCTGAA GTGGTCAGTT

26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT
TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGGA GTCTTAGAA

26151 GCCCCCACC TGCTGTGCAC TTCTAGCGA CTTTGTGCCC ATTAAGTACC
CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201 GCGAATGCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC
CGCTTACGGG AGCGGGCGAA ACCCGGTGA CGATGGAAGA CGTCGATCGG

26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG
TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301 TCTACTGGAG TGTCACCTGC GCTGCAACCT ATGCACCCCG CACCGCTCCC
AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT
ACCAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTGAA
CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCCAATT

26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG
TGAGTGAGGC CCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC

26501 AGGACTACCA CGCCACGAG ATTAGGTTCT ACGAAGACCA ATCCGCCCCG
TCCTGATCGT CCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG AATTACCGC CTGCGTCATT ACCCAGGGCC ACATTCGCG
 GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAGAACC
 26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG
 GGTAAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC
 26651 GACGGGGGGT TACTTGGAC CCCAGTCCG GCGAGGAGCT CAACCCAATC
 CTGCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG
 26701 CCCCCCGCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA
 GGGGGCGCGC GCGTCGGGAT AGTCGTCGTC GCGCCCCGGG AACGAAGGCT
 26751 GGATGGCACC CAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG
 CCTACCGTGG GTTTTTCTTC GACGTCGACG GCGGCGGTGG GTGCCCTGCTC
 26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA
 CTCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT
 26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTCG
 CCTGTACTAC CTCTGACCC TCCTGGATCT GCTCCTTCGA AGGCTCCAGC
 26901 AAGAGGTGTC AGACGAAACA CCGTCACCCCT CGGTGCGATT CCCCTCGCCG
 TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC
 26951 GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC
 CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG
 27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCGG ACCCAACCGT AGATGGGACA
 AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTGGCA TCTACCCTGT
 27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCGGCC GTTAGCCCAA
 GGTGACCTTG GTCCCGGCCA TTCAGGTTCG TCGGCGGCGG CAATCGGGTT
 27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC
 CTCGTTGTG TGCGGTTCC GATGGCGAGT ACCGCGCCCG TGTCTTGCG
 27151 CATAGTTGCT TGCTTGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCCGC
 GTATCAACGA ACGAACGTTG TGACACCCCC GTTGTAGAGG AAGCGGGCGG
 27201 GCTTCTTCT CTACCATCAC GGCCTGGCCT TCCCCGTAA CATCCTGCAT
 CGAAAGAAGA GATGGTAGTG CCGCACCGBA AGGGGGCATT GTAGGACGTA
 27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA
 ATGATGGCAG TAGAGATGTC GGTATGACG TGGCCGCGT CGCCGTCGTT
 27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA
 GTCGTCGCGG GTGTGCTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT
 27351 AAGCCCAAGA AATCCACAGC GCGGCAGCA GCAGGAGGAG GAGCGCTGCG
 TTGCGGTCT TTAGGTGTCG CCGCCGTCGT CGTCTCCTC CTCGCGACGC
 27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT
 AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCCTAAA
 27451 TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG
 AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTTCTC

Figure 26: AC

27501 CTGAAAATAA A CAGGTC TCTGCGATCC CTCACCCGCA GCTGCC TA
 GACTTTTATT TTTGTCCAG AGACGCTAGG GAGTGGGCGT CGACGGACAT
 27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC
 AGTGTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG
 27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT
 AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA
 27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC
 AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTGCGCG GTGTGGGCGG
 27701 GCCAGCACCT GTTGTCAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT
 CGGTCGTGGA CAACAGTCGC GGTAAATCTC GTTCCTTTAA GGTGCGGGA
 27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA
 TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCCGACC TCGACGGGTT
 27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC
 CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG
 27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG
 GGCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC
 27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC
 GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG
 27951 GCTGCCCTGG TGTACCAGGA AAGTCCCCT CCCACCACTG TGGTACTTCC
 CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG
 28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG
 GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC
 28051 CCGGGCGGCTT TCGTCACAGG GTGCGGTGCG CCGGGCAGGG TATAACTCAC
 GCCCGCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG
 28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC
 GACTGTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG
 28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GCGCGCGGCC
 GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG
 28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC
 CGAGAAGTAA GTCCGGAGCA GTCCGTAGG ATTGAGACGT CTGGAGCAGG
 28251 TCTGAGCCGC GCTCTGGAGG CATTGGAAGT CTGCAATTTA TTGAGGAGTT
 AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCTCAA
 28301 TGTGCCATCG GTCTACTTTA ACCCTTCTC GGGACCTCCC GGCCACTATC
 ACACGGTAGC CAGATGAAAT TGGGAAGAG CCCTGGAGGG CCGGTGATAG
 28351 CGGATCAATT TATTCCTAAC TTGACGCGG TAAAGGACTC GCGGACGGC
 GGCTAGTTAA ATAAGGATTG AACTGCGCC ATTTCTGAG CCGCCTCCCG
 28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT
 ATGCTGACTT ACAATTACCC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTT
 CCAGGTGACA GCGCGGTGT TCACGAAACG GCGCGTGAGG CCACTCAAAA
 28501 GCTACTTTGA ATTGCCGAG GATCATATCG AGGGCCCGGC GCACGGCGTC
 CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCGAG
 28551 CCGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTG GGGAGTTTAC
 GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG
 28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG
 GGTGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC
 28651 TGATTTGCAA CTGTCTTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT
 ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA
 28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT
 GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA
 28751 ATCGCCATCC TGTAAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG
 TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC
 28801 CGAACCTTAC CTGCTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG
 GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC
 28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT
 AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA
 28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT
 TGAGGTAGTC TTTTGTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA
 28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT
 CGCAGTGGCC GCGACGTGG TGTGGATGGC GGACTGGCAT TTGTTCTGAA
 29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT
 AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGCTTTGTCC TCCACTCGAA
 29051 AGAAAACCTT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT
 TCTTTTGGGA ATCCCATAAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA
 29101 GAACAATTCA AGCAACTCTA CGGCTATTG TAATTCAGGT TTCTCTAGAA
 CTTGTTAAGT TCGTTGAGAT GCCCGATAAG ATTAAGTCCA AAGAGATCTT
 29151 TCGGGGTGGG GGTATTCTC TGTCTGTGA TTCTCTTTAT TCTTATACTA
 AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT
 29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA
 TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT
 29251 TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT
 AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA
 29301 AATCCTAGGT TTAFTCACCC TTGCGTCAGC CCACGGTACC ACCCAAAGG
 TTAGGATCCA AATGAGTGGG AACGCASTCG GGTGCCATGG TGGGTTTTC
 29351 TGGATTTTAA GGAGCCAGCC TGTAAATGTTA CATTGCGAGC TGAAGCTAAT
 ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29401 GAGTGCACCA CTTATAAA ATGCACCACA GAACATGAAA AGCTGCTTT
 CTCACGTGGT GAGAATATTT TACGTGGTGT CTTGTACTTT TCGACGAATA
 29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTTTATGCT ATTTGGCAGC
 AGCGGTGTTT TTGTTTAAAC CGTTCATACG ACAAATACGA TAAACCGTCG
 29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT
 GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA
 29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT
 TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAAATGGTA
 29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGA
 CATGTACTCG TTTGTCTATAT TCAACACCGG GGGTGTTTTA ACACACCTTT
 29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG
 TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC
 29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA
 CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT
 29751 GGAAAAGAAA ATGCCCTTAAT TTACTAAGTT ACAAAGCTAA TGTCAACCACT
 CCTPTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA
 29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA
 TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT
 29851 ATTAGAATAG GATTTAAACC CCCC GGTCAT TTCCTGCTCA ATACCATTCC
 TAATCTTATC CTAAATTGCG GGGGCCAGTA AAGGACGAGT TATGGTAAGG
 29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA
 GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTGCGGAT GTTGGAACCT
 29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC
 CAGTCCGAAG GACCTACAGT CGTAGACTGA AACC GGTCGT GGACAGGGCG
 30001 GGATTTGTTT CAGTCCAACT ACAGCGACCC ACCCTAACAG AGATGACCAA
 CCTAAACAAG GTCAGGTTGA TGTGCTGCGG TGGGATTGTC TCTACTGGTT
 30051 CACAACCAAC GCGGCCGCGG CTACCGGACT TACATCTACC ACAAATACAC
 GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTATATGTG
 30101 CCCAAGTTTC TGCCCTTGTG AATAACTGGG ATAACCTGGG CATGTGGTGG
 GGGTTCAAAG ACGGAACAG TTATTGACCC TATTGAACCC GTACACCACC
 30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG
 AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC
 30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG
 GACGGATTTT GCGTTTGC GC GGCTGGTGG GTAGATATCA GGGTAGTAAC
 30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC
 ACGATGTGGG TTGTTACTA CTTAGGTAT CTAACCTGCC TGACTTTGTG
 30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT
 TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30351 TTTATATTAC T C CTTGT TCGCCTTTTT TGTGCGTGCT CCAAT C
 AAATATAATG ACTGGGAACA ACGCGAAAA ACACGCACGA GGTGTAACCG
 30401 TCGCGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT
 ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCCGAAG TGTCAGATAA
 30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG
 ACGAAATGCC TAAACAGTGG GAGTCCGAGT AGACGTCGGA GTAGTGACAC
 30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA
 CAGTAGCGGA AATAGGTCAC GTAACGACC CAGACACAG CGAAACGTAT
 30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA
 AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT
 30601 GAATTCCTTA ATTATGAAAT TTAGTGAC TTTTCTGCTG ATTATTGCA
 CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAACGT
 30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA
 GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT
 30701 TGCAGATTCA CTCGTATATG GAATATCCA AGTTGCTACA ATGAAAAAG
 ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTC
 30751 CGATCTTTC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTCT
 GCTAGAAAG CPTCGGACCA ATATACGTTA GTAGAGACAA TACCACAAGA
 30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG
 CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAC GTAACCGACC
 30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCGCGC CCGCTATGCT
 TTGCGTTATC TACGGTACTT GGTGGGTGA AAGGGGCGCG GCGATACGA
 30901 TCCACTGCAA CAAGTTGTTG CCGCGCGCTT TGTCCCAGCC AATCAGCCTC
 AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGTCGG TTAGTCGGAG
 30951 GCCCACCTTC TCCCACCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA
 CCGGTGGAAG AGGGTGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT
 31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC
 CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG
 31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCG CCGAGCAACA GCGCATGAAT
 TCGCGGACGA TCTTCTGCG TCCCGTCGCC GGCTCGTTGT CCGTACTTA
 31101 CAAGAGCTCC AAGACATGGT TAACTTGAC CAGTGCAAAA GGGGTATCTT
 GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTT CCCCATAGAA
 31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC
 AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCAAT TGGTGGCCTG
 31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG
 TGGCGGAATC GATGTTCAAC GGTGGTTTCG CAGTCTTAA CCACAGTAC
 31251 GTGGGAGAAA AGCCATTAC CATAACTCAG CACTCGGTAG AAACCGAAGG
 CACCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31301 CTGCATTAC TCTTGTGTC AAGGACCTGA GGATCTCTGC ACCCTTCTA
 GACGTAAGTG AGTGGAACAG TTCCTGGACT CCTAGAGACG TGGGAATTAAT
 31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAA
 TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTITTT
 31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT
 TATTATTTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA
 31451 TATTACGAG CACCTCCTTG CCTCTCTCC AGCTCTGGTA TTGCAGCTTC
 ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG
 31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC
 GAGGACCGAC GTTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG
 31551 CTGTTCTCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC
 GACAAGGACA GSTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG
 31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG
 CGCGTTCTGG CAGACTTCTA TGAAGTTGG GGCACATAGG TATACTGTGC
 31651 GAAACCGGTC CTCCAAGTGT GCCTTTTCTT ACTCCTCCCT TTGTATCCCC
 CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG
 31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG
 GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC
 31751 AACCTCTAGT TACCTCCAAT GGCACTGCTG CGCTCAAAAT GGGCAACGGC
 TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTTA CCCGTTGCCG
 31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAAATG TAACCACTGT
 GAGAGAGACC TGCTCCGGCC GTTGAATGG AGGGTTTTAC ATTGGTGACA
 31851 GAGCCACCT CTCAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG
 CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC
 31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT
 GTGGGGAGTG TCAATGGAGT CTTGGGGATT GACACCGACG GCGGCGTGGA
 31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCCTAAC
 GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG
 32001 CGTGACAGAC TCCAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT
 GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTACA
 32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT
 GTCTTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGGCTA
 32101 AGCAGTACCC TTAATATCAC TGCCTCACCC CCTCTAACTA CTGCCACTGG
 TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC
 32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC
 ATCGAACCCG TAACTGAAC TTTCTGGGTA AATATGTGTT TTACCTTTTG
 32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT
 ATCCTGATTT CATGCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH

32251 TTGACCGTAG CTGGTCC AGGTGTGACT ATTAATAATA-CTTCCCTCA
 AACTGGCATC GACGAGG TCCACACTGA TAATTATTAT GAAGGAGT
 32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC
 TTGATTTCAA TGACCTCGGA ACCCAAACT AAGTGTTCG TTATACGTTG
 32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA
 AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTTGTC TCGGGAATAT
 32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT
 GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA
 32451 AGGACAGGGC CCTCTTTTTA TAACTCAGC CCACAACCTG GATATTAAC
 TCCTGTCCCG GGAGAAAAT ATTTGAGTCG GGTGTTGAAC CTATAATTGA
 32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAGCTT
 TGTGTTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA
 32551 GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT
 CTCCAATTGG ATTCGTGACG GTTCCCAAC TACAACTGC GATGTCGGTA
 32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA
 TCGGTAATTA CGTCTCTAC CCGAACTTAA ACCAAGTGA TTACGTGTT
 32651 ACACAAATCC CCTCAAAACA AAAATGGCC ATGGCCTAGA ATTTGATTCA
 TGTGTTTAGG GGAGTTTGT TTTAACCAG TACCGGATCT TAACTAAGT
 32701 AACAAAGGCTA TGGTTCCTAA ACTAGGAAC GGCCTAGTT TTGACAGCAC
 TTGTCCGAT ACCAAGGATT TGATCCTGA CCGGAATCAA AACTGTCGTG
 32751 AGGTGCCATT ACAGTAGGAA AAAAAATAA TGATAAGCTA ACTTTGTGGA
 TCCACGGTAA TGTCATCCTT TGTTTTATT ACTATTCGAT TGAACACCT
 32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT
 GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA
 32851 AAACCTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT
 TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA
 32901 TTCAGTTTTG GCTGTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC
 AAGTCAAAAC CGACAATTTT CGTCAACCG AGGTTATAGA CCTTGTCAGG
 32951 AAAGTGCTCA TCTTATTATA AGATTGACG AAAATGGAGT GCTACTAAAC
 TTTACAGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTTG
 33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC
 TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG
 33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG
 ACTTCCGTGT CGGATATGTT TCGACAACC TAAATACGGA TTGGATAGTC
 33101 CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA
 GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTTATGTA ACAGTCAGTT
 33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT
 CAAATGAATT TGCCTCTGTT TTGATTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33201 AAACGGTACA CAAACAG GAGACACAAC TCCAAGTGCA TACTCTTCT
 TTTGCCATGT GTCTTTTGTG CTCTGTGTTG AGGTTACAGT ATGAGATACA
 33251 CATTTTCATG GGAAGTGTCT GGCCACAACCT ACATTAATGA AATATTTGCC
 GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAATTACT TTATAAACGG
 33301 ACATCCTCTT ACACCTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG
 TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC
 33351 TGTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAAT TTCAAGTCAT
 ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA
 33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC
 AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG
 33451 GTACCTTAAT CAAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT
 CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA
 33501 CCCAACACAC AGAGTACACA GTCCCTTCTC CCCGGCTGGC CTTAAAAAGC
 GGGTTGTGTG TCTCATGTGT CAGGAAGAG GGGCCGACCG GAATTTTTCG
 33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT
 TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA
 33601 TTCCTGTGCA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA
 AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTG AGGGGCCCGT
 33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT
 CGAGTGAATT CAAGTACAGC GACAGGTGCA CGACTCGGTG TCCGACGACA
 33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT
 GGTGAAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TCGGGATGTA
 33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA
 CCCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT
 33801 GCGCGCGAAT AACTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC
 CGCGCGCTTA TTGACGACG GCGGCGGCGA GGCAGGACGT CCTTATGTTG
 33851 ATGGCAGTGG TCTCCTCAGC GATGATTGCG ACCGCCCGCA GCATAAGGCG
 TACCGTCACC AGAGGAGTCG CTACTAAGCG TGCGGGGCGT CGTATTCCGC
 33901 CCTTGTCCTC CGGGCACAGC AGCGCACCTT GATCTCACTT AAATCAGCAC
 GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG
 33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG
 TCATTGACGT CGTGTCTGCG TGTATATAA AGTTTTAGGG TGTCACGTTT
 34001 GCGCTGTATC CAAAGCTCAT GCGGGGGACC ACAGAACCCA CGTGGCCATC
 CGCGACATAG GTTTCGAGTA CCGCCCTGGT TGTCTTGGGT GCACCGGTAG
 34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG
 TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC
 34101 ACATAAACAT TACCTCTTTT GGCAATGTTG AATTCACCAC CTCCCGGTAC
 TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34151 CATATAAACC T GATTAAA CATGGCGCCA TCCACCACCA TCCTAATTA
 GTATATTTGG AACTAATT GTACCGCGGT AGGTGGTGGT AGGATTGGT
 34201 GCTGGCCAAA ACCTGCCCGC CGGCTATACA CTGCAGGGAA CCGGGACTGG
 CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC
 34251 AACAAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC
 TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG
 34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT
 CAGTACTATA GTTACAACCG TGTGTGTGCC GTGTGCACGT ATGTGAAGGA
 34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC
 GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTGGG
 34401 ATTCTTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA
 TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT
 34451 CTCACGTTGT GCATTGTCAA AGTGTACAT TCGGGCAGCA GCGGATGATC
 GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG
 34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC
 GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCTCCA TCTGCTAGGG
 34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTGG TCGTAGTGTC
 ATGACATGCC TCACCGCGCT CTGTGGCTC TAGCACAACC AGCATCACAG
 34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCTGAAG CAAAACCAGG
 TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC
 34651 TCGGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC
 ACGCCCGCAC TGTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG
 34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC
 AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGG
 34751 CCTGGCTTCG GGTCTATGT AAACCTCTTC ATGCGCCGCT GCCCTGATAA
 GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGCGCA CGGGACTATT
 34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCTGTC
 GTAGGTGGTG GCGTCTTATT CCGTGTGGGT CGGTTGGATG TGTAAGCAAG
 34851 TGCAGATCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT
 ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA
 34901 TTTTATTATC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAA
 AAAAAATAAG GTTTTCTAAT AGGTTTGGGA GTTTTACTTC TAGATAATTC
 34951 TGAACGCGCT CCCCTCCGGT GCGGTGCTCA AACTCTACAG CCAAAGAACA
 ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTCCTTGT
 35001 GATAATGGCA TTTGTAAGAT GTTGACAAAT GGCTTCCAAA AGGCAAACGG
 CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCGGTTTGCC
 35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC
 GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26 AK

35101 TCTATAAACA TTAGCACC TTCAACCATG CCCAAATAAT TCTCATG
 AGATATTTGT AAGGTCGTGG AAGTTGGTAC GGGTTTATTA AGAGTAGGC

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA
 GGTGGAAGAG TTATATAGAG ATTCTTTAG GGGTTATAAT TCAGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CTTTCAGCCT CAAGCAGCGA
 AACATTTTGA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA
 TAGTACTAAC GTTTTAAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGAGGGC
 TCGCTTGTGA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC
 GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC
 GCGGTCCTTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TCGGTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG
 CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA
 GCTATATTTT ACGTTCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC
 TTTTCTTTT GTGTAGCATC AGTACGAGTA CGTCTATTTT CGTCCATTTC

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC
 AGGCCTTGGT GGTGCTTTT TCTGTGTTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAATAA CAAAAAACA TTAAACATT
 CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA
 TCTTCGACAA GAATGTTGTC CTTTTTGTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA
 GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAAGTG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTCTGTGCC GGAGTCATAA TGTAAGACTC
 TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA
 CCATTGTGT AGTCCAACATA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC
 TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC
 GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTGT GTATTTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA
 ACTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGT

Figure 26 AL

36051 CATAACAGCGC TACAGCG GCAGCCATAA CAGTCAGCCT TACCAG A
 GTATGTCGCG AAGGTGTCGC CGTCGGTATT GTCAGTCGGA ATGGTCATTT
 36101 AAAGAAAACC TATTAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA
 TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT
 36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA
 CAGTGTACACA TTTTTCCTCG GTTCACGTCT CGCTCATATA TATCCTGATT
 36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCCGACG
 TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC
 36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT
 GCTTGATGTC GGGTCTTTGC TTTCCGTTTT TTGGGTGTTG AAGGAGTTTA
 36301 CGTCACTTCC GTTTTCCAC GTTACGTAC TTTCCATTTT AAGAAAATA
 GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT
 36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAACCTA CGTCACCCGC
 GTTAAGGGTT GTGTATGTTT AATGAGCGCG GATTTTGGAT GCAGTGGGCG
 36401 CCGGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA
 GGGCAAGGGT GCGGGGCGCG GTGCAGTGTT TGAGGTGGGG GAGTAATAGT
 PacI

 36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAGA
 ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATCT
 36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCTCATT ATGATTCTTC
 TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG
 36551 TCGCTTCCGG CGGCATCGGG ATGCCCCCGT TGCAGGCCAT GCTGTCCAGG
 AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC
 36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG
 GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC
 36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC
 CTTGGCATT TTTCCGGCGA ACGACCGCAA AAAGGTATCC GAGGCGGGGG
 36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG
 GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC
 36751 ACAGGACTAT AAAGATACCA GGCCTTTCCC CCTGGAAGCT CCCTCGTGCG
 TGTCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC
 36801 CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
 GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG
 36851 CTTCCGGGAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT
 GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA
 36901 TCGGTGTAGG TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT
 AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

36951 TCAGCCCGAC GCGCGCT TATCCGGTAA CTATCGTCTT GAGTCO CC
 AGTCGGGCTG GCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGG TGG
 37001 CCGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT
 GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA
 37051 AGCAGAGCGA GGTATGTAGG CCGTGCTACA GAGTTCTTGA AGTGGTGCGC
 TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG
 37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
 ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT
 37151 AGCCAGTTAC CTTCCGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAAACA
 TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT
 37201 ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGGC
 TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCGTCG TCTAATGCGC
 37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG
 GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC
 37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA
 TCGGAGTCAC CTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT
 37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT
 AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA
 37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT
 CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA
 37451 CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTGC
 GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC
 37501 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA
 ACATCTATTG ATGCTATGCC CTCCGAATG GTAGACCGGG GTCACGACGT
 37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
 TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT
 37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG
 GGTCCGGTCG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC
 37651 CCTCCATCCA GTCTATTAAT TGTGCGCGG AAGCTAGAGT AAGTAGTTCG
 GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC
 37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT
 GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA
 37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
 CAGTGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA
 37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC
 GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTTCG CCAATCGAGG
 37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGAG TGTATCACT
 AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26 A N

37901 CATGGTTATG CACTGTC ATAATTCTCT TACTGTGATG CCATCGTAA
 GTACCAATAC CGTCGTGACG TATTAAGAGA ATGACAGTAC GGTAGGCTT
 37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG
 CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC
 38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC
 ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG
 38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT
 GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA
 38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG
 GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC
 38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG
 ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC
 38201 CGTTTCTGGG TGAGCAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA
 GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT
 38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
 ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA
 38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA
 ATAAC TTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT
 38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCGGAA
 TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT
 38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT
 TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA
 38451 AAAAATAGGC GTATCAGGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA
 TTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

PacI

38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)
 AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 AD

MRKAd5nef MER1063
 (MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

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1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACGCGCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCACAAA TAATAATATC

451 GCGGCGCGGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTCAT
  
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Figure 27A

851 CATGACCTTA TACTTTTCT CTA CTACTTGGCA GTACATCTAC GTATTATTA
GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCTGT

901 TCGCTATTAC CATGGTGATG CGGTTTGGC AGTACATCAA TGGGCGTGGA
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
ACCCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCGG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGG ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCGGCT
CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA
CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGCGCG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
GGCTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCTT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGATGTTC CCGGCGACCT

1551 CCTGTCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC
GGGTCTTCTC CGTCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCT
ATGAAGGGG TGACCGTCTT GATGTGGGG CCGGGCCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCCTGGAG CCCGAGAAGG
GGACTGGAAG CCGACCACGA AGTTCGACCA CCGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC
ACCTCTCCG GTTGCTCCCG CTCTTGTTGA CCGGCGGGT GGGGTACAGG

Figure 27B


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1801 CAGCACGGCA TGGACCC CGAGAAGGAG GTGCTGGAGT GGAGGTGA
      GTCGTGCCGT AGCTCCTGGG GCTCTTCCTC CACGACCTCA CCTCCAAGCT

1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT
      GAGGTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG
      TGTTCTTGAC GATTTCTGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCTC CCCCCTGCCT TCCTTGACCC TGAAGGTGC
      GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCCTT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTTGTC
      GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
      ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCGT CCTGTCGTTC

2101 GGGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC
      CCCCTCTAA CCCTTCTGTT ATCGTCCGTA CGACCCTAC GCCACCCGAG

2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT
      ATACCGGCTA GCGCGCGGCG ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTGT ATCTGTTTGT
      CCCTTCTTA TATATTCCAC CCCAGATAA CATCAAAACA TAGACAAAC

2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTTGTG
      GTCGTCCGCG GCGCGCGTAC TCGTGGTTGA GCAAATACTC TTCGTAACAC

2301 ASCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TCGTCAGAA
      TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCC CGTCCTGCCC GCAAATCTA
      ACACIACCCG AGGTCGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTGGA GACTGCAGCC
      GATGGAACTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCCG CTTCAGCCGC TGCAGCCACC GCGCGCGGGA TTGTGACTGA
      AGGCGCGCGC GAAGTCGGCG ACGTCTGTGG GGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCCGC TTGCAACAG TGCAGCTTCC CGTTCATCCG
      GAAACGAAAG GACTCGGGCG AACGTTTGTG ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC
      GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAACTGG

2601 CGGGAACCTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT
      GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA
      AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT
      TTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

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Figure 27C

2751 TATTTAGGGG TTTTCGCGCG CCGGTAGGCC CGGGACCAGC GGTCTCGGTC
ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTCCAGGAC GTGGTAAAGG TGA CTCTGGA
CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTC ACTGAGACCT

2851 TGTTACAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC
ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA
ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTTCAGTAGC AAGCTGATTG
CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT
GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGATAT GAGATGCATC TTGGACTGTA TTTT TAGGT
CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAATCCAA

3101 GGCTATGTTT CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA
CCGATACAAG GGTGCGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCATG TAGCTTAGAA
GGTCTGTCA CATAGGCCAC GTGAACCCCT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC
CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCTG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCTGGG
GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTCCCCGC CGCCGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA
GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG
AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGCGGTA GTTACCCTCA CAGATTGCA
ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCACGC TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG
AAAGGGTGCG AAAC TCAAGT CTACCCCT AGTACAGATG GACGCCCCGC

3501 ATGAAGAAAA CGGTTTCCGG GTAGGGGAG ATCAGCTGGG AAGAAAGCAG
TACTTCTTTT GCCAAGGCC CCATCCCTC TAGTCGACCC TTCTTTCGTG

3551 GTTCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC
CAAGGACTCG TCGACGCTGA ATGGCGTCG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTATCC
GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTC
GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

Figure 27D

3701 CCTGACCAAA TCCAGAA GCGCTCGCC GCCAGCGAT AGCAGTCTT
 GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA
 3751 GCAAGGAAGC AAAGTTTTC AACGGTTGA GACCGTCCGC CGTAGGCATG
 CGTTCCTTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC
 3801 CTTTTGAGCG TTTGACCAAG CAGTTCAGG CGGTCCCACA GCTCGGTAC
 GAAAACCTCG AACTGGTTC GTCAAGGTCC GCCAGGTGT CGAGCCAGTG
 3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG
 GACGAGATGC CGTAGAGCTA GGTCTATAG AGGAGCAAAG CGCCCAACCC
 3901 GCGGGTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG
 CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC
 3951 TCATGTCTTT CCACGGGCGC AGGGTCTTCG TCAGCGTAGT CTGGGTACG
 AGTACAGAAA GGTGCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC
 4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GTTTGAGGCT
 CACTTCCCCA CGCGAGGCCG GACGCGCGAC CGGTCCACG CGAACTCCGA
 4051 GGTCTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA
 CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT
 4101 GGTAGCATTT GACCATGGTG TCATAGTCCA GCCCTCCGC GCGGTGGCCC
 CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG
 4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG
 AACCGCGCGT CGAACGGGAA CCTCCTCCGC GCGTGCTCC CCGTCACGTC
 4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT
 TGAAACTCC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCCCTA
 4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG
 TCCGTAGGCG CGGCGTCCGG GCGTCTGCC AGAGCGTAAG GTGCTCGGTC
 4301 GTGAGCTCTG GCCGTTCGGG GTCAAAAACC AGGTTTCCCC CATGCTTTT
 CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA
 4351 GATGCGTTTC TTACCTCTGG TTCCATGAG CCGGTGTCCA CGCTCGGTGA
 CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT
 4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCG
 GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCTC GGACAGGAGC
 4451 AGCGGTGTTT CCGGGTCTC CTCGTATAGA AACTCGGACC ACTCTGAGAC
 TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG
 4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC
 TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTACAC CTCCCCATCG
 4551 GGTGTTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG
 CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCACAC TTCTGTGTAC
 4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC
 AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

Figure 27E

4651 GTGACCGGGT CCTGAAG GGGGGCTATA AAAGGGGGTG GGGGCCCTT
 CACTGGGCCA CAAGGACTTC CCCCCGATAT TTTCCCCCAC CCCCAGCGCAA
 4701 CGTCTCACT CTCTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT
 GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA
 4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCAGT
 CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA
 4801 TTCCAAAAAC GAGGAGGATT TGATATTCAC CTGGCCCGCG GTGATGCCTT
 AAGGTTTTTG CTCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA
 4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTGTTGTCA
 ACTCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT
 4901 AGCTTGCTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT
 TCGAACCACC GTTGTCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA
 4951 GGAGCGCAGG GTTGGTTTTT TGTGCGGATC GCGCGCTCC TTGGCCGCGA
 CCTGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCAGG AACCAGCGCT
 5001 TGTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTC GGGAAAGACG
 ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGCGGTAAG CCCTTTCTGC
 5051 GTGGTGCCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGCAG
 CACCACCGA GCAGCCCGTG GTCCACGTGC GCGGTTGCG CCAACACGTC
 5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG
 CCACTGTTC AGTTGCGACC ACCGATGAG AGGCGCATCC GCGAGCAACC
 5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT
 AGGTCTCTC CCGCGCGGG AACGCGCTCG TCTTACCGC ATCCCCAGA
 5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCAGGAG
 TCGACGAGA GCAGGCCCCC CAGACGCAGG TGCCATTCTT GGGGCCCGTC
 5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT
 GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTT AGATCGCGGA
 5301 GCTGCCATGC GCGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA
 CGACGGTACG CCCCCGCGT TCGCGCGCGA GCATACCCAA CTCACCCCT
 5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC
 GGGGTACCGT ACCCCACCCA CTCGCGCTC CCGATGTACG GCGTTTACAG
 5401 GTAAACGTAG AGGGGCTCTC TGAGTATTC AAGATATGTA GGGTAGCATC
 CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG
 5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA
 AAGGTGGCGC CTACGACCGC GCGTGCAITA GCATATCAAG CACGCTCCCT
 5501 GCGAGGAGGT CCGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA
 CGCTCCTCCA GCCCTGGCTC CAACGATGCC CCCCCAGCA GACGAGCCTT
 5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGGACGCT
 CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5601 GGAAGACGTT GCTGGCG TCTGTGAGAC CTACCGCGTC ACGCAGGAG
 CCTTCTGCAA CTTGACCGC AGACACTCTG GATGGCGCAG TGCCTGCTTC
 5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC
 CTCGCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG
 5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT
 CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA
 5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAAACTC TTCGCGGTCT
 CAGGGAAAAA AAAGGTGTCTG AGCGCCAACCT CCTGTTTGAG AAGCGCCAGA
 5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC
 AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG
 5851 TAGCATGTAG AACTGGTTGA CGGCCTGCTA GCGCAGCAT CCCTTTTCTA
 ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT
 5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC
 GCCCATCGCG CATACGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG
 5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT
 CGTTTCCACA GGGACTGGTA CTGAAACTCC ATGACCATAA ACTTCAGTCA
 6001 GTCGTGCGAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGG
 CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC CGGAAAAACC
 6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC
 TTGCGCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG
 6101 GCGCGAGGCA TAAAGTTCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA
 CGCGCTCCGT ATTTCAACGC ACACTACGCC TTCCAGGGC CGTGGAGCCT
 6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTGTA
 TGCCAAACAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT
 6201 TGTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG
 ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACTAC
 6251 GAAGGCAATT TTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG
 CTTCCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC
 6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA
 GGGCACGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTGCTGCT
 6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAG
 TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC
 6401 GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCACTAGAA
 CAGGATTGTA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT
 6451 GGTAAGCGGG TCTTGTTCCT AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT
 CCATTGCCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA
 6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACCT CATGACCAGC
 GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCG

Figure 27G

6551 ATGAAGGGCA () CTGCTT CCCAAAGGCC CCCATCCAAG TATAGG () TC
 TACTTCCCCT GCTCGACGAA GGGTTTCCGG GGGTAGGTTC ATATCCAGAG
 6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG
 ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC
 6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG
 CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACC GA TAACTACACC
 6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA
 ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT
 6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA
 TTTTGACGC GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT
 6801 GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC
 CCAACTGGAC TGCTGGCGCG GTTTCCTTCG TCTCACCTT AACTCGGGG
 6851 TCGCCTGGCG GGTTTGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG
 AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC
 6901 ACCGTCTGCG TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC
 TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGGCGCG
 6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTGGAG CTTGATGACA
 CGCTCGGGT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACACTGT
 7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG
 TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCGAGT
 7051 GTCAGCGGGG AGCTCCTGCA GGTTTACCTC GCATAGACGG GTCAGGGCGC
 CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG
 7101 GGGCTAGATC CAGGTGATAC CTAATTCCA GGGGCTGGTT GGTGGCGGCG
 CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCAGACAA CCACCGCCGC
 7151 TCGATGGCTT GCAAGAGGCC GCATCCCGCG GCGCGACTA CCGTACCGCG
 AGCTACCGAA CGTTCCTCCG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC
 7201 CCGCGGGCGG TGGGCGCGG GGGTGTCTT GGATGATGCA TCTAAAAGCG
 GCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC
 7251 GTGACGCGGG CGAGCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGA
 CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCGAGGCTT GGGCGGCCCT
 7301 GAGGGGGCAG GGGCACGTG GCGCCGCGCG CCGGCAGGAG CTGGTGCTGC
 CTCCCCGTC CCCGTGCAGC CGCGCGCGC GCCCGTCTC GACCAGGAG
 7351 GCGCGTAGGT TGCTGGCGAA CGCGACGAG CCGCGGTTGA TCTCCTGAAT
 CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA
 7401 CTGGCGCCTC TGCCTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG
 GACCGCGGAG ACGCACTTCT GCTGCCCCGG CCACTCGAAC TTGGACTTTC
 7451 AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAA
 TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCGG GACCGCGTTT

Figure 27H

7501 ATCTCCTGCA CCTCCTGA GTTGTCTTGA TAGGCGTCTA GGGCGTCTA
 TAGAGGACGT GAGGAGACT CAACAGAACT ATCCGCTAGA GCCGGTCTT
 7551 CTGCTCGATC TCTCCTCCT GGAGATCTCC GCGTCCGGCT CGTCCACGG
 GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC
 7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG
 ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCGCAAC
 7651 AGGCCTCCCT CGTTCAGAC GCGGCTGTAG ACCACGCCCC CTTCGGCATC
 TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGCTCGGGG GAAGCCGTAG
 7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA
 CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT
 7751 AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGCGG
 TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC
 7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC
 CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG
 7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA
 CAACTATAGG GGGTTCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT
 7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC
 GCCGCTTCAA CTTTGTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG
 7951 TCCAGAAGAC GGATGAGCTC GCGGACAGTG TCGCGCACCT CGCGCTCAAA
 AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGA GCGGAGTTT
 8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT
 CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCAGA
 8051 CCCCTTCTTC TTCTTCTGGC GCGGCTGGGG GAGGGGGGAC ACGGCGGCGA
 GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCGCCGCT
 8101 CGACGGCGCA CCGGAGGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG
 GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC
 8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTCTCTG CCGGGGCGCA
 CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCGCCGCGT
 8201 GTTGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CCGGGGCTG
 CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCAACC GCGCCCGC
 8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTGTGT
 GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA
 8301 AGGTACTCCG CCGCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG
 TCCATGAGGC GCGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC
 8351 AAAACCTCTC GAGAAAGGCG TCTAACCACT CACAGTCGCA AGGTAGGCTG
 TTTTGGAGAG CTCTTCCGC AGATTGGTCA GTGTACGCGT TCCATCCGAC
 8401 AGCACCGTGG CCGGCGGCG CCGGCGGCG TCGGGGTTGT TTCTGGCGGA
 TCGTGGCACC GCGCGCGTC GCGCGCGC AGCCCCAACA AAGACCGCT

Figure 27I

8451 GGTGCTGCTG AATGTGAAT TAAAGTAGGC GGTCTTGAGA CGGCGGCG
 CCACGACGAC TACTACATTA ATTCATCCG CCAGAACTCT GCCGCCTACC
 8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG
 AGCTGTCTTC GTGGTACAGG AAGCCAGGCC GGACGACTTA CGCGTCCGGC
 8551 TCGCCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA
 AGCCGGTACG GGGTCCGAAG CAAAAGTGA GCCGCGTCCA GAAACATCAT
 8601 GTCTTGCAATG AGCCTTTCTA CCGGCACCTC TTCTTCTCCT TCCTCTTGTC
 CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG
 8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG
 GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC
 8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG
 ACCCGGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC
 8751 AAGCAGGGCT AGGTGCGCGA CAACGCGCTC GGCTAATATG GCCTGCTGCA
 TTCGTCCCGA TCCAGCCGCT GTTGCGCGAG CCGATTATAC CGGACGACGT
 8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT
 GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA
 8851 GCGCCCGTGT TGATGGTGTG AGTGCAGTTG GCCATAACGG ACCAGTTAAC
 CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG
 8901 GGTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG
 CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC
 8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGACCCAG GTACTGGTAT
 GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA
 9001 CCCACCAAAA AGTGCAGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT
 GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA
 9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT
 CCGGCCCGCA GGCCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA
 9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC
 TCTACATGGA CCTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG
 9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC
 CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG
 9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT
 GTACCAGCCC TCGGAGACCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA
 9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT
 TCTGGCACGT TTTCTCTCG GACATTGCCC CGTGAGAAGG CACCAGACCA
 9301 GGATAAATTC GCAAGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT
 CCTATTAAAG CGTTCCATA GTACCGCTG CTGGCCCCAA GCTCGGGGCA
 9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCCGG TGTCGAACCC
 TAGGCCGGCA GCGGGCACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9401 AGGTGTGCGA CAGACAA CGGGGGAGTG CTCCTTTTGG CTTCTTTA
 TCCACACGCT GCACTCTGTT GCCCCTCAC GAGGAAAACC GAAGGAAGGT
 9451 GGCAGCGCGG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG
 CCGCGCCGCC GACGACGCGA TCGAAAAAAC CGGTGACCGG CCGCGCTCGC
 9501 TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCTGTAG
 ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC
 9551 CCGGAGGGTT ATTTTCCAAG GGTGAGTCTG CGGGACCCCC GGTTCGAGTC
 GGCCTCCCAA TAAAGGTTTC CCAACTCAGC GCCCTGGGGG CCAAGCTCAG
 9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCCTCCC CGTCATGCAA
 AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AACCGGAGGG GCAGTACGTT
 9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT
 CTGGGCGGAA CGTTTAAGGA GCCCTTTGTC CCTGCTCGGG GAAAAACGA
 9701 TTTCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG
 AAAGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC
 9751 CCGCAAGAGC AAGAGCAGCG GCAGACATGC AGGCACCCCT CCCCTCCTCC
 GCCGTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG
 9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG
 ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC
 9851 ATTACGAACC CCCGCGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG
 TAATGCTTGG GGGCGCCGCG GCCCGGGCGG TGATGGACCT GAACCTCCTC
 9901 GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG
 CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTT
 9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC
 CCACGTGAC TTGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG
 10001 TGTTTCGCGA CCGCGAGGGA GAGGAGCCCC AGGAGATGCG GGATCGAAAG
 ACAAGAGCCT GCGCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC
 10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT
 AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA
 10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCAGGATT AGTCCCGCGC
 CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG
 10151 GCGCACACGT GCGGCGCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG
 CGCGTGTGCA CCGCGGCGCG CTGGACCATT GCGGTATGCT CGTCTGCCAC
 10201 AACCAGGAGA TTAACCTTCA AAAAGCTTT AACAACCACG TGCGTACGCT
 TTGCTCCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA
 10251 TGTGCGCGCG GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG
 ACACCGCGCG CTCCTCCACC GATATCTGA CTACGTAGAC ACCCTGAAAC
 10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GCGCGAGCTG
 ATTCGCGCGA CCTCGTTTTG GGTATATCGT TCGCGAGTA CCGCGTCGAC

Figure 27K

10351 TTCCTTATAG TGCACAG CAGGGACAAC GAGGCATTCA GGGATGCT
 AAGGAATATC ACCTCGTGTC GTCCCTGTTG CTCCGTAAGT CCCTACGCGA
 10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA
 CGATTTGTAT CATCTCGGGC TCCCGGCGAC CGACGAGCTA AACTATTTGT
 10451 TCCTGCAGAG CATACTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG
 AGGACGTCCT GTATCACCAC GTCTCGCGT CGAACTCGGA CCGACTGTTC
 10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCC
 CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC
 10551 CAAGATATAC CATACCCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG
 GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCTC CATTTCTAGC
 10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC
 TCCCAAGAT GTACGCGTAC CCGGACTTCC ACGAATGGAA CTCGCTGCTG
 10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG
 GACCCGCAAA TAGCGTTGCT CCGCTAGGTG TTCCGGCACT CGCACTCGGC
 10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC
 CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCCCGGG
 10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCTTA CTTTGACGCG
 ACCGACCGTG CCCGTCGCGC CTATCTCTCC GGCTCAGGAT GAAACTCGGC
 10801 GGGCTGACC TGCCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG
 CCGGCACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC
 10851 GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTCGGCG
 CCGGCTTGA CCCGACCGCC ACCGTGGGCG CCGCGACCGG TTGCAGCCGC
 10901 GCGTGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG
 CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC
 10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG
 ATGATTCGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC
 11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA
 CGCCACGCCG GCGCGACGCT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT
 11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC
 GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG
 11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG
 GACTGCGCAA GGCCGTCGTC GCGTCCGCT TGGCCGAGAG GCGTTAAGAC
 11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCG ACGCACGAGA AGGTGCTGGC
 CTTGCCACC AGGGCCGCGC GCGTTGGGG TGCGTGTCT TCCACGACCG
 11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG
 CTAGCATTG CGCGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC
 11251 GCCTGGTCTA CGACGCGCTG CTTACGCGCG TGGCTCGTTA CAACAGCGGC
 CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCGC

Figure 27L

11301 AACGTGCAGA CCTGGA CCGGCTGGTG GGGGATGTGC GCGAGGTT
 TTGCACGTCT GGTGGACCT GGCCGACCAC CCCCTACACG CGCTCCGGCA
 11351 GGCGCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG
 CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC
 11401 CACTAAACGC CTTCTGAGT ACACAGCCCC CCAACGTGCC GCGGGGACAG
 GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTGACACGG CGCCCTGTG
 11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGA CTGAGAC
 CTCCTGATGT GGTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG
 11501 ACCGCAAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA
 TGGCGTTTCA CTCCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT
 11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAACTTG
 CATCTGTTC GGACGTCTGG CATTTGGACT CGGTCCGAAA GTTTTGAAC
 11601 CAGGGGCTGT GGGGGGTGCG GGCTCCACA GCGGACCGCG CGACCGTGTC
 GTCCCCGACA CCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG
 11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT
 ATCGAACGAC TCGGGTTGA GCGCGGACAA CGACGACGAT TATCGCGGGA
 11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACTAGG TCACTTGCTG
 AGTGCTGTG ACCGTGCGAC AGGGCCCTGT GTATGGATCC AGTGAACGAC
 11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT
 TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA
 11801 CCAGGAGATT ACAAGTGTCA GCCGCGCGCT GGGGCAGGAG GACACGGGCA
 GGTCTCTAA TGTTCACAGT CCGCGCGCGA CCCCCTCTC CTGTGCCCGT
 11851 GCCTGGAGGC AACCCTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC
 CGGACCTCCG TTGGGATTTG ATGGACGACT GGTGGCCGC CGTCTTCTAG
 11901 CCCTCGTTGC ACAGTTTAAA CAGCGAGGAG GAGCGCATTT TCGCTACGT
 GGGAGCAACG TGTCAAATTT GTCGCTCCTC CTCGCGTAAA ACGCGATGCA
 11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCCAGCG
 CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TCGGGGTGCG
 12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACC GGCGAT GTATGCCTCA
 ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATA CGGAGT
 12051 AACC GGCGCT TTATCAACCG CTAATGGAC TACTTGATC GCGCGGCCGC
 TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGCG
 12101 CGTGAACCCC GAGTATTTC CCAATGCCAT CTTGAACCCG CACTGGCTAC
 GCACTTGGGG CTCATAAAGT GGTACGGTA GAACTTGGGC GTGACCGATG
 12151 CGCCCCCTGG TTCTACACC GGGGGATTG AGGTGCCCGA GGTAACGAT
 GCGGGGGACC AAAGATGTGG CCCCCTAAGC TCCACGGGCT CCCATTGCTA
 12201 GGATTCCTCT GGGACGACAT AGACGACAGC GTGTTTTCCC CGCAACCGCA
 CCTAAGGAGA CCCTGCTGTA TCTGCTGTCG CACAAAAGGG GCGTTGGCGT

Figure 27 M

12251 GACCCCTGCTA GACCTGCAAC AGCGCGAGCA GGCAGAGGCG GCGCTGCTA
 CTGGGACGAT CACGTTG TCGCGCTCGT CCGTCTCCGC CGCGACCTT
 12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC
 TCCTTTTCGAA GCGGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG
 12351 CCGCGGTCAG ATGCTAGTAG CCCATTTCCTA AGCTTGATAG GGTCTCTTAC
 GCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG
 12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA
 GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT
 12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATT
 TGTGAGCGA CGACGTCCGC GTGCGCTTT TTTTGACGG AGGCCGTAA
 12501 CCCAACACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC
 GGGTGTGTC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTCTG
 12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC
 CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG
 12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG
 CAGTTTCCGT GCTGGCAGTC GCGCCAGACC ACACCCTCCT GCTACTGAGC
 12651 GCAGACGACA GCAGCGTCCT GGATTGCGGA GGGAGTGGCA ACCCGTTTGC
 CGTCTGCTGT CGTCGAGGA CCTAAACCT CCCTACCGT TGGGCAAACG
 12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAACAAAAA AAAAAGCATG
 CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTCTT TTTTTCGTAC
 12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTTCT
 TACGTTTTAT TTTTGTAGTG GTTCCGGTAC CGTGGCTCGC AACCAAAGA
 12801 TGTATTTCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC
 ACATAAGGGG AATCATACGC CGCGCGCCGC TACATACTCC TTCCAGGAGG
 12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG
 AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTACCCGC CGCCGCGACC
 12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC
 CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAACACGG AGGCGCCATG
 12951 CTGCGGCCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC
 GACGCCGGAT GGCCCCCTC TTTGTGCTAG GCAATGAGAC TCAACCGTGG
 13001 CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG
 GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTGTGTC AGTTGCCTAC
 13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC
 ACCGTAGGGA CTTGATGCTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG
 13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA
 TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT
 13151 TCTTGACGAC CGGTGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA
 AGAACTGCTG GCCAGCGTGA CCCCCTGCT GGACTTTTGG TAGGACGTAT

Figure 27N

13201 CCAACATGCC AATGTTGAAC GAGTTCATGT TTACCAATAA GTTAAATG
 GGTGTACGG TTTCACTTG CTCAAGTACA AATGGTTATT CAAATTGTC
 13251 CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA
 GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT
 13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCGA GGGCAACTAC TCCGAGACCA
 TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT
 13351 TGACCATAGA CCTTATGAAC AACCGGATCG TGGAGCACTA CTTGAAAGTG
 ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACCTTCAC
 13401 GGCAGACAGA ACGGGGTCTT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC
 CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACCTGTG
 13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG
 GCGGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC
 13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA
 CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAGTAAAA CGACGGTCCT
 13551 TGCGGGGTGG ACTTCACCCA CAGCCGCGCT AGCAACTTGT TGGGCATCCG
 ACGCCCCACC TGAAGTGGGT GTCGCGGGAC TCGTTGAACA ACCCGTAGGC
 13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG
 GTTCGCCGTT GGAAGGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC
 13651 AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCCTA CCAGGCGAGC
 TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG
 13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GCGCGAGGCG GCAGCAACAG
 AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC
 13751 CAGTGGCAGC GCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC
 GTCACCGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTTACG
 13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC
 TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG
 13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC
 TGTGCCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG
 13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA
 GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT
 13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC
 AGTTTGGGGA CTGTCTCCTG TCGTCTTTG CGTCAATGTT GGATTATTCC
 14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAACTA
 TTACTGTCTG GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT
 14051 CGGCACCCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACCTCTG
 GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC
 14101 ACGTAACTG CGGCTCGGAG CAGGTCTACT GGTGTTGCC AGACATGATG
 TGCATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14151 CAAGACCCCG TTTTCCG CTCCACGCGC CAGATCAGCA ACTTTC
 GTTCTGGGGC ACTGGAAGGC GAGGTGCGCG GTCTAGTCGT TGAAAGGCTA
 14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC
 CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG
 14251 AGGCGGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG
 TCCGCGCAGT GAGGGTTGAG TAGGCGCTCA AATGGAGACA CTGGGTGCAC
 14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCCAC
 AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CGCGCGGGCG CTCGGGGGTG
 14351 CATCACCACC GTCAGTAAA ACGTTCTGTC TCTCAGAT CACGGGACGC
 GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGTCTA GTCCCTGCG
 14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC
 ATGGCGACGC GTTGTCTAG CCTCCTCAGG TCGCTCACTG GTAATGACTG
 14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC
 CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG
 14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTC AGCAAGCATG TCCATCCTTA
 CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT
 14551 TATCGCCAG CAATAACACA GGCTGGGGCC TGCCTTCCC AAGCAAGATG
 ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC
 14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCAGCG
 AAACCGCCCC GGTCTTTCGC GAGGCTGGTT GTGGGTACG CGCACGCGCC
 14651 GCACTACCGC GCGCCCTGGG GCGCGCACA ACAGCGCCCGC ACTGGGCGCA
 CGTGATGGCG CGCGGGACCC CGCGCTGTT TCGCCGCGC TGACCCCGT
 14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC
 GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG
 14751 ACGCCACCGC CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT
 TCGGGGTGCG GCGGTGGTCA CAGGTGTAC CTGCGCCGGT AAGTCTGGCA
 14801 GGTGCGCGGA GCGCGCGCT ATGCTAAAT GAAGAGACCG CGGAGGCGCG
 CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC
 14851 TAGCACGTGC CCACCGCCGC CGACCCGCA CTGCCGCCA ACGCGCGGCG
 ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TCGCGCGCGC
 14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCGGACGGG CGGCCATGCG
 CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCGGTACGC
 14951 GCGCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA
 CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT
 15001 GCGCAGGAGC GCGCGCGCA GCAGCGCGG CCATTAGTGC TATGACTCAG
 CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAAATCAG ATACTGAGTC
 15051 GGTGCGAGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCTGCG
 CCAGCGTCCC CGTTGCACAT AACCACGCG CTGAGCCAAT CGCCGACGC

Figure 27P

15101 CGTGCCCGTG CCCCCCGCC CCCC GCGCAA CTAGATTGCA TGAATAA
 GCACGGGCAC GCGGGGCGG GGGGCGCGTT GATCTAACGT TCTTTT
 15151 ACTTAGACTC GACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA
 TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT
 15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC
 CGATACAGGT TCGCGTTTTA GTTCTTCTC TACGAGGTCC AGTAGCGCGG
 15251 GGAGATCTAT GSCCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA
 CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGCTT
 15301 AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAAGTTGAC
 TCGATTTCGC CCAGTTTTTC TTTTCTTTC TACTACTACT ACTTGAAGTG
 15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG
 CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCAC
 15401 GAAAGGTGCA CGCGTAAAC GTGTTTTGCG ACCCGGCACC ACCGTAGTCT
 CTTTCCAGCT GCGCATTTTG CACAAAACGC TGGGCGGTGG TGGCATCAGA
 15451 TTACGCCCGG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG
 AATGCGGGCC ACTCGCGAGG TGGGCGTGGA GTTCGCGCA CATACTACTC
 15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA
 CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTGCTCG CGGAGCCCCCT
 15551 GTTTCCTTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG
 CAAACGGATG CCTTTCGCGG TATTCTGTA CGACCGCAAC GCGGACCTGC
 15601 AGGGCAACCC AACACCTAGC CTAAAGCCCC TAACACTGCA GCAGGTGCTG
 TCCCGTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC
 15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCTTAAAGC GCGAGTCTGG
 GGGCGCGAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTCG CGCTCAGACC
 15701 TGACTTGCCA CCCACCGTGC AGCTGATGCT ACCCAAGCGC CAGCGACTGG
 ACTGAACCGT GGGTGGCAGC TCGACTACCA TGGGTTTCGCG GTCGCTGACC
 15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC
 TTCTACAGAA CCTTTTTTAC TGGCACCTTG GACCCGACCT CGGGCTCCAG
 15801 CGCGTGGCGC CAATCAAGCA GGTGGCGCCG GGAAGTGGCG TGCAGACCGT
 GCGCACGCGG GTTAGTTTCGT CCACCGCGGC CTTGACCCGC ACGTCTGGCA
 15851 GGACGTTTCA ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG
 CCTGCAAGTC TATGGGTGAT GGTCAATCGT GTCATAACGG TGGCGGTGTC
 15901 AGGGCATGGA GACACAAAGC TCCCCGCTG CCTCAGCGGT GCGGATGCC
 TCCCGTACCT CTGTGTTTC AGGGGCCAAC GGAGTCGCCA CCGCTTACGG
 15951 GCGGTGCAGG CGGTGCTGC GCGCGCTCC AAGACCTCTA CGGAGGTGCA
 CGCCACGTCC GCCAGCGAGC CCGGCGCAGG TTCTGGAGAT GCTTCCACGT
 16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGCGC CCGCGCCGTT
 TTGCTGGGC ACCTACAAAG CGCAAAGTC GGGGCGCGC GCGCGGCCAA

Figure 27A

16051 CGAGGAAGTA CCGGCGCC AGCGCGCTAC TGCCCGAATA TGCCCTAATT
 GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA
 16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG
 GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC
 16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC
 TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG
 16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT
 CAGCGGCAGC GGTGCGGCAC GAACGGGGCT AAAGGCACGC GTCCACCGA
 16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCAG
 GCGCTTCCTC CGTCCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC
 16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT
 GTAGCAAATT TTCGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA
 16351 GCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG
 CGGCGGAGGC AAAGGCCAC GGCCTAAGG CTCCTTCTTA CGTGGCATCC
 16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGCACCA
 TCCCGTACC GGCCGCTGCC GGAATGCCCG CCGTACGCAG CACGCGTGGT
 16451 CCGCGCGCGG CGCGCGTCGC ACCGTGCGAT GCGCGCGCGT ATCCTGCCCC
 GGCCGCCGCC GCGCGCAGCG TGGCAGCGTA CCGCGCGCCA TAGGACGGGG
 16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA
 AGGAATAAGG TGAATAGCGG CGCCGCTAAC CCGCGCACGG GCCTTAACGT
 16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTAATAACAA GTTGCATGTG
 AGGCACCGGA ACGTCCGCGT CTCGTGACT AATTTTGT CAACGTACAC
 16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCTGTAA
 CTTTATTAGT TTATTTTTCA GACCTGAGAG TCGGAGCGAA CCAGGACATT
 16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCGCGCGACA
 GATAAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT
 16701 CCGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA
 GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT
 16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT
 ACTCGCCACC GCGGAAGTCG ACCCGAGCG ACACCTCGCC GTAATTTTAA
 16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC
 AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCTGT
 16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTT CAACAAAAGG
 TCCGGTCTAC GACTCCCTAT TCAACTTCT CGTTTTAAAG GTTGTTTTCC
 16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGA CCTGGCCAAC
 ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG
 16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT
 GTCCGTCACG TTTTATTCTA ATTGTCATTG GAACTAGGGG GGGGAGGGCA

Figure 27R

17001 AGAGGAGCCT CCGGCCG TGGAGACAGT GTCTCCAGAG GGGCGT
 TCTCTCGGA GGTGGCCGGC ACCTCTGTCA CAGAGGTCTC CCGGACCGC
 17051 AAAAGCGTCC GCGCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC
 TTTTCGCAGG CCGGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG
 17101 GAGCCTCCCT CGTACGAGGA GGCATAAAG CAAGGCCTGC CCACCACCCG
 CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTCCGGACG GGTGGTGGGC
 17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA
 AGGGTAGCGC GGTACCGAT GGCCTCACGA CCCGGTCGTG TGTGGGCATT
 17201 CGCTGGACCT GCCTCCCCC GCGACACCC AGCAGAAACC TGTGCTGCCA
 GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT
 17251 GGGCCGACCG CCGTTGTTGT AACCCGTCTT AGCCGCGCGT CCCTGCGCCG
 CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACCGCGC
 17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC
 GCGGCGGTG CAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG
 17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC
 TTTGCTGTGA CTGTGCTAG CACCCAGACC CCCACGTAG GGACTTCGCG
 17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC
 GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG
 17451 CATGTGCGCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG
 GTACAGCGGC GGTCTCCTCG ACGACTCGGC GCGCGCGCGG CGAAAGGTTT
 17501 ATGGCTACCC CTTGATGAT GCCGCACTGG TCTTACATGC ACATCTCGGG
 TACCGATGGG GAAGCTACTA CCGCGTCACC AGAATGTACG TGTAGAGCCC
 17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGAG TTTGCCCGCG
 GGTCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC
 17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG
 GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC
 17651 GCGCCTACCG ACCACGTGAC CACAGACCGG TCCCAGCGTT TGACGCTGCG
 CCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC
 17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT
 CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA
 17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC
 AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG
 17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC
 AAATGTAGG CCGCGCACGA CCTGTCCCCG GGATGAAAT TCGGGATGAG
 17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG
 ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGACGC
 17901 AATGGGATGA AGCTGCTACT GCTCTGAAA TAAACCTAGA AGAAGAGGAC
 TTACCCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCTG

Figure 275

17951 GATGACAACG ACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAA
 CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTGTGAGT
 18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA
 GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT
 18051 TTCAAATAGG TGTGGAAGGT CAAACACCTA AATATGCCGA TAAACATTT
 AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAA
 18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA
 GTTGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT
 18151 TCATGCAGCT GGGAGAGTCC TAAAAAGAC TACCCCAATG AAACCATGTT
 AGTACGTCGA CCCTCTCAGG ATTTTCTCTG ATGGGGTTAC TTTGGTACAA
 18201 ACGGTTTCATA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT
 TGCCAAGTAT ACGTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA
 18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTT
 CATTTGCTTG TTTTACCTTT CGATCTTCA GTTCACCTTT ACGTTAAAAA
 18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG
 GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTT
 18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGA CACTCATATT
 ACCATAACAT GTCACCTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA
 18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA
 AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT
 18451 ACAATCTATG CCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTA
 TGTTAGATAC GGGTTGTCCG GATTAAATGA ACGAAAATCC CTGTTAAAT
 18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC
 AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG
 18551 CAAGCATCGC AGTTGAATGC TGTGTAGAT TTGCAAGACA GAAACACAGA
 GTTCGTAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT
 18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT
 CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA
 18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTAGAATT
 AAAGATACAC CTTAGTCCGA CAACTGTGCA TACTAGGTCT ACAATCTTAA
 18701 ATTGAAAATC ATGGAAGTGA AGATGAACTT CCAAATTACT GCTTTCCACT
 TAACTTTGTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA
 18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAACAG
 CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTG
 18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAT
 CAGTCCCTTT ACCTACCCCT TTTCTACGAT GTCTTAAAAG TCTATTTTAA
 18851 GAAATAAGAG TTGGAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA
 CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18901 CCTGTGGAGA A~~TC~~CTGT ACTCCAACAT AGCGCTGTAT TTGCCC~~A~~
 GGACACCTCT TTAAGGACA TGAGTTGTA TCGCGACATA AACGGGCTGT
 18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC
 TCGATTTTCAT GTCAGGAAGG TTGCATTTT AAAGACTATT GGGTTTGTGG
 19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA
 ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT
 19051 CATTAACCTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC
 GTAATTGGAA CCTCGTGCGA CCAGGGAACCT GATATACCTG TTGCAGTTGG
 19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG
 GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC
 19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTCTT
 CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA
 19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA
 ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT
 19251 ACTTCAGGAA GGATGTTAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC
 TGAATCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG
 19301 CTAAGGGTTG ACGGAGCCAG CATTAAGTTT GATAGCATTT GCCTTTACGC
 GATTTCCCAAC TGCTTCGGTC GTAATTCAA CTATCGTAAA CGGAAATGCG
 19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC
 GTGGAAGAAG GGGTACCGGG TGTGTGGCG GAGGTGCGAA CTCCGGTACG
 19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC
 AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG
 19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT
 TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA
 19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCTTC ACGCGCCTTA
 GGGGAGGGCG TTGACCGGCC GAAAGGCGCC GACCCGGAAG TCGCGGAAT
 19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC
 TCTGATTCTT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG
 19601 TACTCTGGCT CTATACCTTA CCTAGATGGA ACCTTTTACC TCAACCACAC
 ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG
 19651 CTTTAAGAAG GTGGCCATTA CTTTGACTC TTCTGTCAGC TGGCCTGGCA
 GAAATTCTTC CACCGGTAAT GGAACTGAG AAGACAGTCG ACCGGACCGT
 19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC
 TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTAAATTCGC GAGTCAACTG
 19751 GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCTCT
 CCCCTCCCAA TGTGCAACG GGTACATTG TACTGGTTTC TGACCAAGGA
 19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC
 CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA C GACCGC ATGTACTCCT TCTTTAGAAA CTTCCAC
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGGA TGATACTAAA TACAAGGACT ACCAACAGGT
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTCGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT
GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGCGGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA
TATCCGTTCT GCGTCAACT GTCGTAATGG GTCTTTTCA AAGAAACGCT

20101 TCGCACCCCTT TGGCGCATCC CATTCTCCAG TAACCTTATG TCCATGGGCG
AGCGTGGGAA ACCCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAATC CGCCACGCG
GTGAGTGTCT GGACCCGGT TTGGAAGAGA TCGGTTGAG CGGGTGCGC

20201 CTAGACATGA CTTTGTAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA
GATCTGTACT GAAAACTCCA CCTAGGGTAC CTGCTCGGGT GGAAGAAAT

20251 TGTTTTGT TT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG
ACAAAACAA CTTCAGAAAC TGCACCAGG ACACGTGGTC GCGTGGCGC

20301 GCGTCATCGA AACCGTGATC CTGCGCACGC CTTCTCGGC CGGCAACGCC
CGCAGTAGCT TTGGCACATG GACGCGTGC GGAAGAGCCG GCCGTTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC
TGTTGTATTT CTTGCTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAAA GATCTTGGTT GTGGGCCATA
TCACTCGTCC TTGACTTTTC GTACAGTTT CTAGAACCA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA
TCGAGCGGAC GCGGTATCAG TTATGCCGCG CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTG
ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCTGCGC CGTAGCGCCA TTGCTTCTTC CCCGACCGC
TCATGCTCAG TGAGGACGCG GCATCGCGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC
ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC
GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27V.

20801 CCCAAACTCC C GATCAC AACCCACCA TGAACCTTAT TACCGG A
 GGGTTTGAGG GTACCTAGTG TTGGGGTGGT ACTTGGAATA ATGGCCCCAT
 20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TCGCTCGCAA
 GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT
 20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA
 GGTCTCTTGTG GAGATGTGCA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT
 20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTGTGCA CTTGAAAAAC
 CGGTGTCAAG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG
 21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA
 TACATTTTTA TTACATGATC TCTGTGAAAG TTATTCCGT TIACGAAAAAT
 21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG
 AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC
 21101 TTTAAAAATC AAAGGGGTTT TGCCGCGCAT CGCTATGCGC CACTGGCAGG
 AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC
 21151 GACACGTTCG GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC
 CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG
 21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA
 GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT
 21251 CCAACGCGTT TAGCAGGTCG GCGCGCGATA TCTTGAAGTC GCAGTTGGGG
 GGTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAACTTCAG CGTCAACCCC
 21301 CCTCCGCCCT GCGCGCGCGA GTTGCATAC ACAGGGTTGC AGCACTGGAA
 GGAGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT
 21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTGCGAGA
 GTGATAGTCG CGGCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT
 21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC
 AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG
 21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCCGAGGT TTGAGTTGCA
 AAACCATCGA CGGAAGGGTT TTTCCCGCGC ACGGGTCCGA AACTCAACGT
 21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCCGTC TGGGCGTTAG
 GAGCGTGCCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC
 21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAGC CACCTGAGCC
 CTATGTGCGG GACGTATTTT CGGAAC TAGA CGAATTTTCG GTGGACTCGG
 21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT
 AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGCC TTTTGACTAA
 21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTGCGTCG GTGTTGGAGA
 CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT
 21701 TCTGCACCAC ATTTCCGGCC CACCGGTTCT TCACGATCTT GGCTTTGCTA
 AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27W

21751 GACTGCTCCT TCGCGCG CTGCCCCGTTT TCGCTCGTCA CATUATC
CTGACGAGGA AGTCGCGCGC GACGGGCAAA AGCGAGCAGT GTAGGTAAAG

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT
TTAGTGACAG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA

21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCCTGGGC
GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTCGCGT CGGGCACCCG

21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG
AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TCGGACGTC

21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGSTCAGCT
CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA

22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGATAC GCCCGCCAGA
CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT

22051 GCTTCCACTT GGTACGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC
CGAAGGTGAA CCAGTCCGTC ATCAAACCTC AAGCGGAAAT CTAGCAATAG

22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC
GTGCACCATG AACAGGTAGT CCGCGCGCGC TCGGAGGTAC GGAAGAGGG

22151 ACGCAGACAC GATCGGCACA CTCAGCGGCT TCATCACCGT AATTTCACTT
TGCGTCTGTG CTAGCCGTGT GAGTCGCCCCA AGTAGTGGCA TTAAAGTGAA

22201 TCCGCTTCGC TGGGCTCTTC CTCTTCTCT TCGCTCCGA TACCACGCGC
AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGC GCG

22251 CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTC
GTGACCCAGC AGAAGTAAGT CCGCGGCGTG ACACGCGAAT GGAGGAAACG

22301 CATGCTTGAT TAGCACCGGT GGGTTGCTGA AACCACCAT TTGTAGCGCC
GTACGAATA ATCGTGGCCA CCCAACGACT TTGGGTGGTA AACATCGCGG

22351 ACATCTTCTC TTTCTTCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG
TGTAAGAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC

22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGCAATGG
CGCGAGCCCG AACCTCTTC CCGCAAGAA AAAGAAGAAC CCGCGTTACC

22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC
GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG

22501 AGCGCGTCTT GTGATGAGTC TTCTCTGTC TCGGACTCGA TACGCCGCCT
TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGCGCGA

22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGG
GTAGGCGAAA AAACCCCGC GGGCCCTCC GCCGCGCTG CCCCTGCCCC

22601 ACGACACGTC CTCCATGCTT GGGGACGTC GCGCCGCACC GCGTCCGCGC
TGCTGTGACG GAGGTACCAA CCCCCTGACG CGCGCGGTGG CGCAGGCGCG

22651 TCGGGGGTGG TTTGCGGCTG CTCTCTTCC CGACTGGCCA TTCTCTCTC
AGCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27X

22701 CTATAGGCAG AAGATCA TGGAGTCAGT CGAGAAGAAG GACAGC
 GATATCCGTC TTTTCTAGT ACCTCAGTCA GCTCTTCTTC CTGTCCGATT
 22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG
 GCGGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC
 22801 CCTACCACCT TCCCCGTCGA GGCACCCCGC CTTGAGGAGG AGGAAGTGAT
 GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA
 22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG
 ATAGCTCGTC CTGGGTCCAA AACATTGCTT TCTGCTGCTC CTGGCGAGTC
 22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG
 ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTCGCTCT CCGTTTGCTC
 22951 GAACAAGTCG GCGGGGGGGA CGAAGGCAT GCGACTACC TAGATGTGGG
 CTTGTTACGC CCGCCCCCTC GCTTTCGTA CCGCTGATGG ATCTACACCC
 23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG
 TCTGCTGCAC GACAACTTCG TAGACGTCGC GGTACGCGG TAATAGACGC
 23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC
 TGCGAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG
 23101 CTTGCCTACG AACGCCACCT ATTCTCACCG CCGGTACCCC CCAAACGCCA
 GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTTGCGGT
 23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT
 TCTTTTGCCG TGTACGCTCG GGTGGGGCGC GGAGTTGAAG ATGGGGCATA
 23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTT CCAAACTGC
 AACGGCACGG TCTCCACGAA CCGTGGATAG TGTAGAAAAA GGTTTTGACG
 23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT
 TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTCGTCGA
 23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG
 CCGGAACGCC GTCCCGCGAC AGTATGACT ATAGCGGAGC GAGTTGCTTC
 23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC
 ACGGTTTTTA GAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCGTTTG
 23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTGGT
 CGAGACGTTG TCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA
 23451 GGAACTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG
 CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC
 23501 AGGTCACCCA CTTTGCCCTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG
 TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC
 23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG
 TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC
 23601 GGATGCAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCASTTGGCG
 CCTACGTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y

23651 ACGAGCAGCT ACGCTGG CTTCAAACGC GCGAGCCTGC CGACTT G
 TGCCTGTCGA TCGCGCGACC GAAGTTTGGC CGCTCGGACG GCTGAACCTC
 23701 GAGCGACGCA AACTAATGAT GGCCGCACTG CTCGTTACCG TGGAGCTTGA
 CTCGCTGCGT TTGATTACTA CCGCGCTCAC GAGCAATGGC ACCTCGAACT
 23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG
 CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC
 23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG
 TTTGTAACGT GATGTGGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTT
 23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA
 TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAACGT
 23901 CGAAAACCGC CTGGGGCAA ACCTGCTTCA TTCCACGCTC AAGGGCGAGG
 GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC
 23951 CGCGCCCGCA CTACGTCGCG GACTGCGTTT ACTTATTCT ATGCTACACC
 GCGCGGCGCT GATGCAGGCG CTGACGCAA TGAATAAAGA TACGATGTGG
 24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT
 ACCGCTGCGC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTTGGA
 24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTGAAGGAC CTATGGACGG
 GTTCCTCGAC GTCTTTGACG ATTTGTTTTT GAACTTCCTG GATACCTGCC
 24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC
 GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCTGTGA GTAAAAGGGG
 24151 GAACGCCTGC TTA AACCCCT GCAACAGGGT CTGCCAGACT TCACCACTCA
 CTTGCGGACG AATTTTGGGA CGTTGTCCA GACGGTCTGA AGTGGTCAGT
 24201 AAGCATGTTG CAGAACTTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT
 TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA
 24251 TGCCCCCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAGTAC
 ACGGGCGGTG GACGACACGT GAAGGATCGC TGAACACCG GTAATTCATG
 24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC
 GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG
 24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG
 GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC
 24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC
 CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG
 24451 CTGGTTTGCA ATTGCGAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT
 GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA
 24501 TGAGCTGCAG GGTCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTGGA
 ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT
 24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT
 TTGAGTGAGG CCCCACACCC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 272

24601 GAGGACTACC AC~~CC~~CCACGA GATTAGGTTT TACGAAGACC AATCCCG~~CC~~
 CTCCTGATGG TCGGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG

24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG
 CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC

24701 GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG
 CGGTAAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAGA CGATGCTTTC

24751 GGACGGGGGG TTTACTTGGA CCCCCAGTCC GGCAGGAGC TCAACCCAAT
 CCTGCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTGA

24801 CCCCCGCGC CCGCAGCCCT ATCAGCAGCA GCCGCGGGCC CTTGCTTCCC
 GGGGGCGGC GCGCTCGGA TAGTCGTCGT CCGCGCCCGG GAACGAAGGG

24851 AGGATGGCAC CCAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA
 TCCTACCGTG GGT~~TTTT~~CTT CGACGTCGAC GCGGGCGGTG GGTGCCTGCT

24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGT~~TTT~~TGGAC GAGGAGGAGG
 CCTCCTATG ACCCTGTCAG TCCGTCTCCT CCAAACCTG CTCCTCCTCC

24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC
 TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG

25001 GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTGCGAT TCCCCTCGCC
 CTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG

25051 GGCGCCCCAG AAATCGGCAA CCGGTTCAG CATGGCTACA ACCTCCGCTC
 CCGCGGGGTC TTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG

25101 CTCAGGCGCC GCCGGCACTG CCGGTTCGCC GACCCAACCG TAGATGGGAC
 GAGTCCGCGG CGGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG

25151 ACCACTGGAA CCAGGGCCCG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA
 TGGTGACCTT GTTCCCGGCC ATTCAGGTTT GTGCGCGGCG GCAATCGGGT

25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAACG
 TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTCTTGC

25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTGCCCCG
 GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG

25301 CGCTTTCTTC TCTACCATCA CGGCGTGGCC TTCCCCGTA ACATCCTGCA
 GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT TGTAGGACGT

25351 TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA
 AATGATGGCA GTAGAGATGT CCGGTATGAC GTGGCCCGCG TCGCCGTCGT

25401 ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC
 TGTGTCGCC GGTGTGCTT CGTTCCGCT GGCTATCGT TCTGAGACTG

25451 AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC
 TTTGGGGTTC TTTAGGTGTC GCCGCCGTCG TCGTCTCCT CCTCGCGACG

25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT
 CAGACCGCGG GTTGTGTTGG CATAGCTGGG CGCTCGAATC TTTGTCTTAA

Figure 27. AA

25551 TTTCCCACTC TCTTGCTAT ATTTCAACAG AGCAGGGGCC AAGAACA
 AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTTCT
 25601 GCTGAAAATA AAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT
 CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA
 25651 ATCACAAAAG CGAAGATCAG CTTCCGGCGCA CGCTGGAAGA CGCGGAGGCT
 TAGTGTTTTT GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCTCCGA
 25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT
 GAGAAGTCAT TTATGACGCG CCACTGAGAA TTCTGATCA AAGCGCGGGA
 25751 TTCTCAAAAT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG
 AAGAGTTTAA ATTCCGCGCT TTGATGCAGT AGAGGTCGCC GGTGTGGGCC
 25801 CGCCAGCACC TGTGTCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC
 GCGTTCGTGG ACACAGTCG CCGTAATACT CGTTCCTTTA AGGTCGCGG
 25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGTCGCCA
 ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT
 25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT
 TCTGATGAGT TGGGCTTAT TGTGTACTC GCGCCCTGGG GTGTACTATA
 25951 CCCGGGTCAA CGGAATACGC GCCCACCAGG ACCGAATTCT CCTGGAACAG
 GGGCCAGTT GCCTTATGCG CCGGTGGCTT TGGCTAAGA GGACCTTGTC
 26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC
 CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG
 26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC
 GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG
 26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAATCAGG GGCGCAGCTT
 GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA
 26151 GCGGGCGGCT TTCGTCACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA
 CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT
 26201 CCTGACAATC AGAGGGCGAG GTATTAGCT CAACGACGAG TCGGTGAGCT
 GGACTGTTAG TCTCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA
 26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC
 GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG
 26301 CGCTCTTCAT TCACGCTCG TCAGGCAATC CTAATCTGC AGACCTCGTC
 GCGAGAAGTA AGTCCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG
 26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT
 GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA
 26401 TTGTGCCATC GGTCTACTTT AACCCCTTCT CCGGACCTCC CGGCCACTAT
 AACACGGTAG CCAGATGAAA TTGGGAAGA GCCCTGGAGG GCCGCTGATA
 26451 CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG
 GGCTTAGTTA AATAAGGATT GAACTGCGC CATTTCTGA GCCCCTGCC

Figure 27 AB

26501 CTACGACTGA A TAAGTG GAGAGGCAGA GCAACTGCGC CTGAAA C
 GATGCTGACT TACAATTCAC CTCCTCCGTCT CGTTGACGCG GACTTTGTGG
 26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT
 ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA
 26601 TGCTACTTTG AATTGCCCCG GGATCATATC GAGGGCCCCG CGCACGGCGT
 ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA
 26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCC TAGCCTGATT CGGGAGTTTA
 GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT
 26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTTCCTACT
 GGGTCGCGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA
 26751 GTGATTTGCA ACTGTCCTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA
 CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT
 26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TAAAAATATA CTGGGGCTCC
 AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG
 26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG
 ATAGCGGTAG GACATTGCG GTGGCAGAAG TGGGCGGGTT CGTTTGTTTC
 26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA
 CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT
 26951 GTTTCACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC
 CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGAGAG GCTCGAGTCG
 27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCGGG AACGTACGAG
 ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGCCCC TTGCATGCTC
 27051 TGGCTACCCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT
 ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA
 27101 TTTTCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT
 AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTGTG CTCCACTCGA
 27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA
 ATCTTTTGGG AATCCCATAA TCCGTTTCC GCGTCGATGA CACCCCAAAT
 27201 TGAACAATT CAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA
 ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT
 27251 ATCGGGGTTG GGGTTATTCT CTGTCTGTG ATTCTCTTTA TTCTTATACT
 TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA
 27301 AACGCTTCTC TGCTTAAGGC TCGCCGCTG CTGTGTGCAC ATTTGCATTT
 TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA
 27351 ATTGTGAGCT TTITAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA
 TAACAGTCGA AAAATTGCG ACCCCAGCGG TGGGTTCTAC TAATCCATGT
 27401 TAATCCTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG
 ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTTC

Figure 27AC

27451 GTGGATTTTA A GGCAGC CTGTAATGTT ACATTGCGAG CTGAAG A
 CACCTAAAAT TCCTCGGTG GACATTACAA TGTAAGCGTC GACTTCGATT
 27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA
 ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGTACTT TTCGACGAAT
 27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG
 AAGCGGTGTT TTTGTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC
 27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA
 GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT
 27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA
 ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT
 27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA
 ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT
 27751 AACACTGGCA CTTTCGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT
 TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA
 27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG
 CCAGACATGG GATGAGATAT AATTATGTT TTCGTCTGCG TCGAAATAAC
 27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTACCAC
 TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG
 27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT
 ATTGACGAAA TGAGCGACGA ACGTTTGTG TAAGTTTTTC AATCGTAATA
 27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATT
 TTAATCTTAT CCTAAATTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG
 28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA
 GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTGCGGA TGTGGAAC
 28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG
 TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC
 28101 CGGATTTGTT CAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA
 GCCTAAACAA GGTGAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT
 28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA
 TGTGTTGGTT CGCGCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT
 28201 CCCCAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG
 GGGGTTCAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC
 28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT
 CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA
 28301 GCTGCCTAAA GCGCAAACGC GCGCGACCAC CCATCTATAG TCCCATCATT
 CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA
 28351 GTGCTACACC CAAACAATGA TGAATCCAT AGATTGGACG GACTGAAACA
 CACGATGTGG GTTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28401 CATGTTCTTT TTTACAG TATGATTAAA TGAGACATGA TTCCTC
GTACAAGAAA AGAGAATGTC ATACTAATTT ACTCTGTACT AAGGAGCTCA

28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG
AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC

28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT
GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA

28551 TTGCTTTACG GATTTGTAC CCTCACGCTC ATCTGCAGCC TCATCACTGT
AACGAAATGC CTAAACAGTG GGAGTCCGAG TAGACGTCGG AGTAGTGACA

28601 GGTTCATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT
CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA

28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT
TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCGATATCG ACTCGAAGAA

28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTCTGCT GATTATTTGC
TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG

28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAA GACATATATC
TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG

28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTGCTAC AATGAAAAA
TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACCATG TTACTTTT

28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC
CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG

28901 TCCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG
ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC

28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCGCG CCCGCTATGC
CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG

29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCAGC CAATCAGCCT
AAGGTGACGT TGTTCACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA

29051 CGCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG
CGGGGTGGA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC

29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG
TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCCTA ATAATGTCTC

29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCAGCAAC AGCGCATGAA
GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT

29201 TCAAGAGCTC CAAGACATCG TTAACCTGCA CCAGTGCAA AGGGGTATCT
AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTCACGTTT TCCCATAGA

29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA
AAACAGAGCA TTTCGTCCGG TTTCAGTGA TGCTGTCAAT ATGGTGGCCT

29301 CACCGCCTTA GCTACAAGTT GCCAACCAG CGTCAGAAAT TGGTGGTCAT
GTGGCGGAAT CGATGTTCAA CGTTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27 A E

29351 GGTGGGAGAA A CATT A CCATAACTCA GCACTCGGTA GAAACC G
 CCACCTCTT TTCGGGTAAT GGTATGAGT CGTGAGCCAT CTTTGGCTTC
 29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT
 CGACGTAAGT GAGTGAACA GTTCTGGAC TCCTAGAGAC GTGGGAATAA
 29451 AAGACCTCTGT GCGGTCTCAA AGATCTTATT CCCTTAACT AATAAAAAA
 TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTT
 29501 AATAATAAAG CATCACTTAC TAAAAATCAG TTAGCAAATT TCTGTCCAGT
 TTATTATTTC GTAGTGAATG AATTTIAGTC AATCGTTTAA AGACAGGTCA
 29551 TTATTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT
 AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA
 29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCACTTTCCT
 GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA
 29651 CCTGTTCTCG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG
 GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC
 29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC
 GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG
 29751 GGAAACCGGT CCTCCAAC TGCTTTTCT TACTCCTCCC TTTGTATCCC
 CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG
 29801 CCAATGGGTT TCAAGAGAGT CCCCTGGGG TACTCTCTTT GCGCCTATCC
 GGTTACCCAA AGTTCTCTCA GGGGACCCC ATGAGAGAAA CGCGGATAGG
 29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG
 CTTGGAGATC AATGGAGGTT ACCGTACGAA CCGGAGTTTT ACCCGTTGCC
 29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAAT GTAACCACTG
 GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGTTTTTA CATGGTGAC
 29951 TGAGCCCACC TCTCAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT
 ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA
 30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC
 CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GCGGCGGTGG
 30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA
 AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT
 30101 CCGTGCACGA CTCCAAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG
 GGCACGTGCT GAGGTTTGAA TCGTAACGCT GGGTTCCTGG GGAGTGTAC
 30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA
 AGTCTTCCTT TCGATCGGGA CGTTGTAGT CCGGGGGAGT GGTGGTGGCT
 30201 TAGCAGTACC CTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG
 ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC
 30251 GTAGCTTGGG CATTGACTTG AAAGAGCCA TTTATACACA AAATGGAATA
 CATCGAACCC GTAACGAAC TTTCTCGGT AATATGTGT TTTACCTTTT

Figure 27 AF

30301 CTAGGACTAA ACGGGGC TCCTTTGCAT GTAACAGACG ACCTAA C
 GATCCTGATT TCGCCCG AGGAAACGTA CATTGTCTGC TGGATTG
 30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC
 AAACCTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG
 30401 AAATAAAGT TACTGGAGCC TTGGGTTTTC ATTACACAAGG CAATATGCAA
 TTTGATTTC AATGACCTCGG AACCCAAAAC TAAGTGTTC GTTATACGTT
 30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT
 GAATTACATC GTCCTCCTGA TTCCTAATA AGAGTTTTGT CTGCGGAATA
 30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC
 TGAACACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG
 30551 TAGGACAGGG CCCTCTTTTT ATAACTCAG CCCACAACCT GGATATTAAC
 ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTGAA CCTATAATTG
 30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAACAATT CAAAAAGCT
 ATGTTGTTTC CGGAAATGAA CAAATGTCGA AGTTGTGTA GGTTTTTCGA
 30651 TGAGGTTAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA
 ACTCCAATTG GATTCGTGAC GGTTCCCCAA CTACAAACTG CGATGTCGGT
 30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA
 ATCGGTAATT ACGTCCTCTA CCCGAACTTA AACCAAGTGG ATTACGTGGT
 30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTG
 TTGTGTTTAG GGGAGTTTTG TTTTAAACCG GTACCGGATC TTAAACTAAG
 30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA
 TTTGTTCGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACGTGCTG
 30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG
 GTCCACGGTA ATGTCATCCT TTGTTTTAT TACTATTCGA TTGAACACC
 30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC
 TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTACGTC TCTTCTACG
 30951 TAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG
 ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC
 31001 TTTCAGTTTT GGCTGTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT
 AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTATAG ACCTTGTCOA
 31051 CAAAGTGCTC ATCTTATTAT AAGATTGAC GAAATGGAG TGCTACTAAA
 GTTTCACGAG TAGAATAATA TTCTAAACTG CTTTACCTC ACGATGATT
 31101 CAATTCCTTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA
 GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT
 31151 CTGAAGGCAC AGCCTATACA AACGCTGTG GATTTATGCC TAACCTATCA
 GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT
 31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCAGTCA
 CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG

31251 AGTTTACTTA AAGGAGACA AACTAAACC TGTAACACTA ACCATTAC
 TCAAATGAAT TTGCCTCTGT TTTGATTGG ACATTGTGAT TGGTAATGTG
 31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG
 ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTCACG TATGAGATAC
 31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC
 AGTAAAAGTA CCCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAACG
 31401 CACATCCTCT TACACTTTTT CATACATTGC CCAAGAATAA AGAATCGTTT
 GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTTATT TCTTAGCAA
 31451 GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA
 CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT
 31501 TTTTTCATT AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC
 AAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG
 31551 CGTACCTTAA TCAAACTCAC AGAACCTTAG TATCAACCT GCCACCTCCC
 GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG
 31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCAGCTGG CCTTAAAAAG
 AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGCCGACC GGAATTTTTC
 31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG
 GTAGTATAGT ACCCATTGTC TGTATAAGAA TCCACAATAT AAGGTGTGCC
 31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC
 AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCG
 31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG
 TCGAGTGAAT TCAAGTACAG CGACAGGTG ACGACTCGGT GTCCGACGAC
 31801 TCCAACTTGC GGTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA
 AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT
 31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC
 ACCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTCG
 31901 AGCGCGCGAA TAAACTGCTG CCGCCGCCGC TCCGTCTGTC AGGAATACAA
 TCGCGCGCTT ATTTGACGAC GCGCGCGCG AGGCAGGACG TCCTTATGTT
 31951 CATGGCAGTG GTCTCCTCAG CGATGATTCG CACCGCCCGC AGCATAAGGC
 GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG
 32001 GCCTTGTCTT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA
 CGGAACAGGA GGCCCGTGTG GTCCGCTGGG ACTAGAGTGA ATTTAGTCGT
 32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA
 GTCATTGACG TCGTGTCTGT GTGTATAAC AAGTTTTAGG GTGTCACGTT
 32101 GCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT
 CCGCGACATA GGTTCGAGT ACCGCCCTG GTGTCTTGGG TGACCGGTA
 32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG
 GTATGGTGTG CCGGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32201 GACATAAACA TCTCTTT TGGCATGTTG TAATTCACCA CCTCCC A
 CTGTATTTGT AATGGAGAAA ACCGTACAAC ATTAAGTGGT GGAGGGCCAT
 32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC
 GGTATATTTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTTGG
 32301 AGCTGGCCAA AACCTGCCCG CCGGCTATAC ACTGCAGGGA ACCGGGACTG
 TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC
 32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT
 CTTGTTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA
 32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC
 GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG
 32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGGAAACAACC
 AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTGG
 32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA
 GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT
 32551 ACTCACGTTG TGCATTGTCA AAGTGTACA TTCGGGCAGC AGCGGATGAT
 TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA
 32601 CCTCCAGTAT GGTAGCGCGG GTTTCGTCT CAAAAGGAGG TAGACGATCC
 GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTCCTCC ATCTGCTAGG
 32651 CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTG GTCGTAGTGT
 GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAAAC CAGCATCACA
 32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTGAA GCAAAACCAAG
 GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC
 32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG
 CACGCCCCGA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC
 32801 CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC
 GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG
 32851 CCCTGGCTTC GGGTTCATG TAAACTCCTT CATGCGCCGC TGCCCTGATA
 GGGACCGAAG CCCAAGATAC ATTTGAGGAA GTACGCGCGC ACGGGACTAT
 32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTGCTT
 TGTAGGTGGT GGCCTCTTAT TCGGTGTGGG TCGGTGGAAT GTGTAAGCAA
 32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT
 GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA
 33001 TTTTTTTATT CCAAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA
 AAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT
 33051 GTGAACGCGC TCCCTCCCG TGGCGTGGTC AAATCTACA GCCAAAGAAC
 CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG
 33101 AGATAATGGC ATTTGTAAGA TGTGCACAA TGGCTTCCAA AAGGCAAACG
 TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33151 GCCCTCACGT GTGGAC GTAAAGGCTA AACCCCTTCAG GTGGAATTC
 CGGGAGTGCA GGCACCTG CATTTCGAT TTGGGAAGTC CCACTTTCAG
 33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC
 GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG
 33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC
 CGGTGGAAGA GTTATATAGA GATTCGTTTA GGGCTTATAA TTCAGGCCGG
 33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG
 TAACATTTT AGACGAGGTC TCGCGGGAGG TGGGAAGTCG AGTTCGTGCG
 33351 AATCATGATT GCAAAAATTC AGGTTCCTCA CAGACCTGTA TAAGATTCAA
 TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT
 33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG
 TTCGCCCTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC
 33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC
 GGTGCACTTG TATTAGCAGC TCCAGACGTG CCTGGTCGCG CCGGTGAAGG
 33501 CCGCCAGGAA CCATGACAAA AGAACCACCA CTGATTATGA CACGCATACT
 GCGGTCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA
 33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG
 GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTGGAACA ACGTACCCGC
 33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC
 CGCTATATTT TACGTTCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG
 33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG
 TTTTTCCTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC
 33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG
 GAGGCCCTTG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC
 33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT
 GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA
 33801 TAGAAGCCTG TCTTACAACA GAAAAACAA CCCTTATAAG CATAAGACGG
 ATCTTCGGAC AGAATGTTGT CCTTTTGTG GGAATATTC GTATTCTGCC
 33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA
 TGATGCCGCT ACGGCCGCAC TGGCATTITT TTGACCAAGT GCACTAATTT
 33901 AAGCACCACC GACAGCTCCT CGGTCAATGC CGGAGTCATA ATGTAAGACT
 TTCGTGGTGG CTGTGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA
 33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG
 GCCATTTGTG TAGTCCAAC TAAAGTAGCC AGTCACGATT TTTCGCTGGC
 34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC
 TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG
 34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC
 GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTGT TGTATTTGTG

Figure 27A5

34101 CTGAAAAACC CACCTGCCTA GGCAAAATAG CACCCCTCCGG CTGCGACGTA
 GACTTTTTTG GACCGAT CCGTTTTATC GTGGGAGGGC GAGGTCCT
 34151 ACATACAGCG CTTCACAGC GGCAGCCATA ACAGTCAGCC TTACCAAGTAA
 TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT
 34201 AAAAGAAAAC CTATTAAAA AACACCACTC GACACGGCAC CAGCTCAATC
 TTTCTTTTG GATAATTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG
 34251 AGTCACAGTG TAAAAAGGG CCAAGTGAG AGCGAGTATA TATAGGACTA
 TCAGTGTCAC ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT
 34301 AAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC
 TTTTACTGC ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG
 34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCCTCAAA
 CGCTTGGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT
 34401 TCGTCACTTC CGTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAACT
 AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGTAA ATTCTTTTGA
 34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG
 TGTTAAGGGT TGTGTATGTT CAATGAGCGG GGATTTTGGG TGCAGTGGGC
 34501 CCCCCTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC
 GGGGCAAGGG TCGGGGCGC GSTGCAGTGT TTGAGGTGGG GGAGTAATAG

PacI

 34551 ATATTGGCTT CAATCCAAA TAAGGTATAT TATTGATGAT GTTAATTAAG
 TATAACCGAA GTTAGGTTTT ATTCCATATA ATAATACTA CAATTAATTC
 34601 AATTCGGATC TCGACGCGA GGCTGGATGG CCTTCCCAT TATGATTCTT
 TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA
 34651 CTCGCTTCCG GCGGCATCGG GATGCCCCGCG TTGCAGGCCA TGCTGTCCAG
 GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC
 34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA
 CGTCCATCTA CTGCTGGTAG TCCCTGTCTGA AGTTCCGGTC GTTTTCCGGT
 34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC
 CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG
 34801 CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
 GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG
 34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
 CTGTCCGTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG
 34901 GCTCTCTGT TCCGACCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
 CGAGAGGACA AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG
 34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG
 GGAAGCCCTT CGCACCSCGA AAGAGTATCG AGTGCGACAT CCATAGAGTC
 35001 TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCG
 AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC

Figure 27 AK

35051 TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAAC
AAGTCGGGCT GGCGACCGCG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACCTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC
TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTCTGC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT
CGTCTTTTTT TCCTAGAGTT CTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG TCATGAGATT
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA
TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
ACTCATTGTA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGCAATAA
TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCTGTC AACTTTATCC
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCAGG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTGCGTCTC CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGCGCT CACAATAGTG

Figure 2 AL

36001 TCATGGTTAT ~~CG~~AGCACTG CATAATTCTC TTACTGTCAT GCCATC ~~TA~~
AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAACA CGGGATAATA
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAGAGTGC TCATCATTGG AAAACGTTCT
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
AGCCCCGCTT TTAGAGTTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA

36251 GTAACCCACT CGTGACCCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAGGGA
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTCCT

36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA
TATTCCTGCT GTGCCTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTACCTA
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTTCG TCTTCAAGAA TTGGATCCGA
ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM

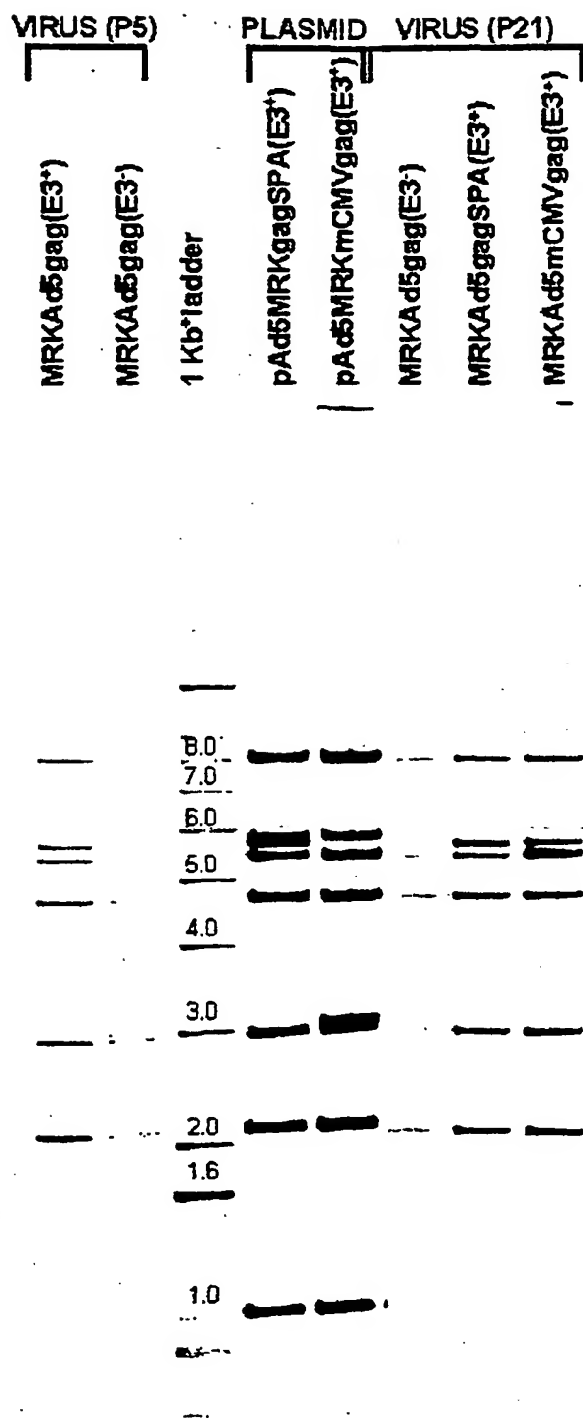


FIGURE 28

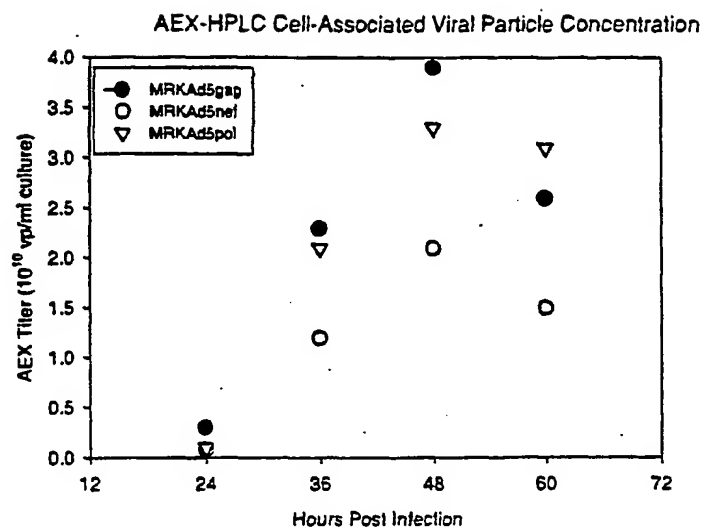


FIGURE 29A

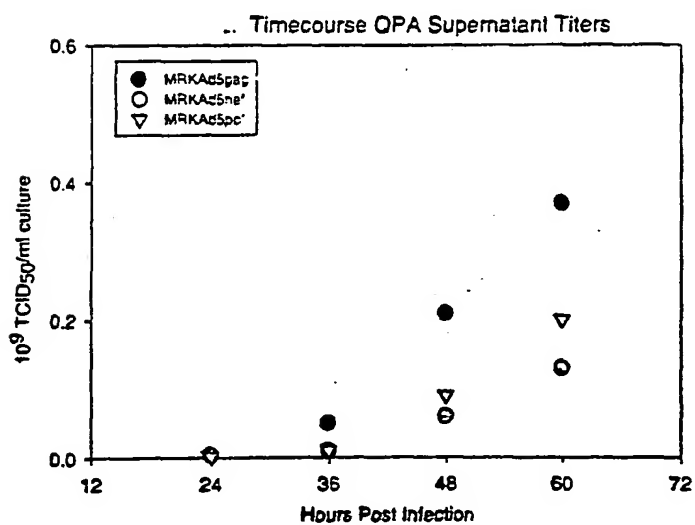


FIGURE 29B

atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly 1 5 10 15	48
gca gtc ttc gtt tcg ccc agc gag atc tcc att gtg tgg gcc tcc agg Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg 20 25 30	96
gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu 35 40 45	144
ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly 50 55 60	192
tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys 65 70 75 80	240
gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys 85 90 95	288
att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala 100 105 110	336
gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val 115 120 125	384
cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr 130 135 140	432
ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu 145 150 155 160	480
gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp 165 170 175	528
ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln 180 185 190	576
atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu 195 200 205	624
cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro 210 215 220	672
agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile 225 230 235 240	720
ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys 245 250 255	768

Figure 30A'

agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro 260 265 270	816
acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp 275 280 285	864
tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln 290 295 300	912
gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn 305 310 315 320	960
cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu 325 330 335	1008
gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys 340 345 350	1056
gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr 355 360 365	1104
atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys 370 375 380	1152
tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala 385 390 395 400	1200
ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met 405 410 415	1248
aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro 420 425 430	1296
tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro 435 440 445	1344
aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr 450 455 460	1392
ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala 465 470 475 480	1440
tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482 Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37) 485 490	

Figure 30 B

Figure 31

IFN- γ Secretion against Gag 20-aa pool from CD3⁺ T cells of Monkey PBMCs

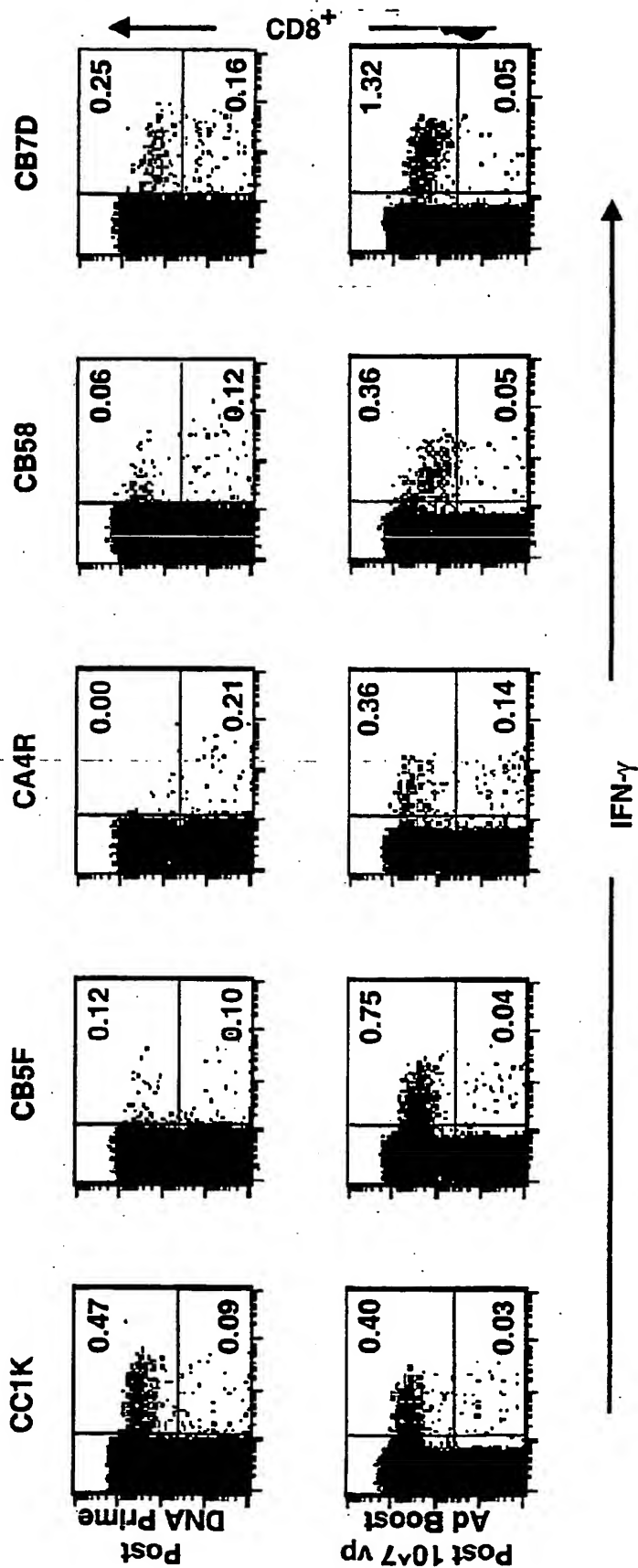


FIGURE 32

Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost

Immunizations

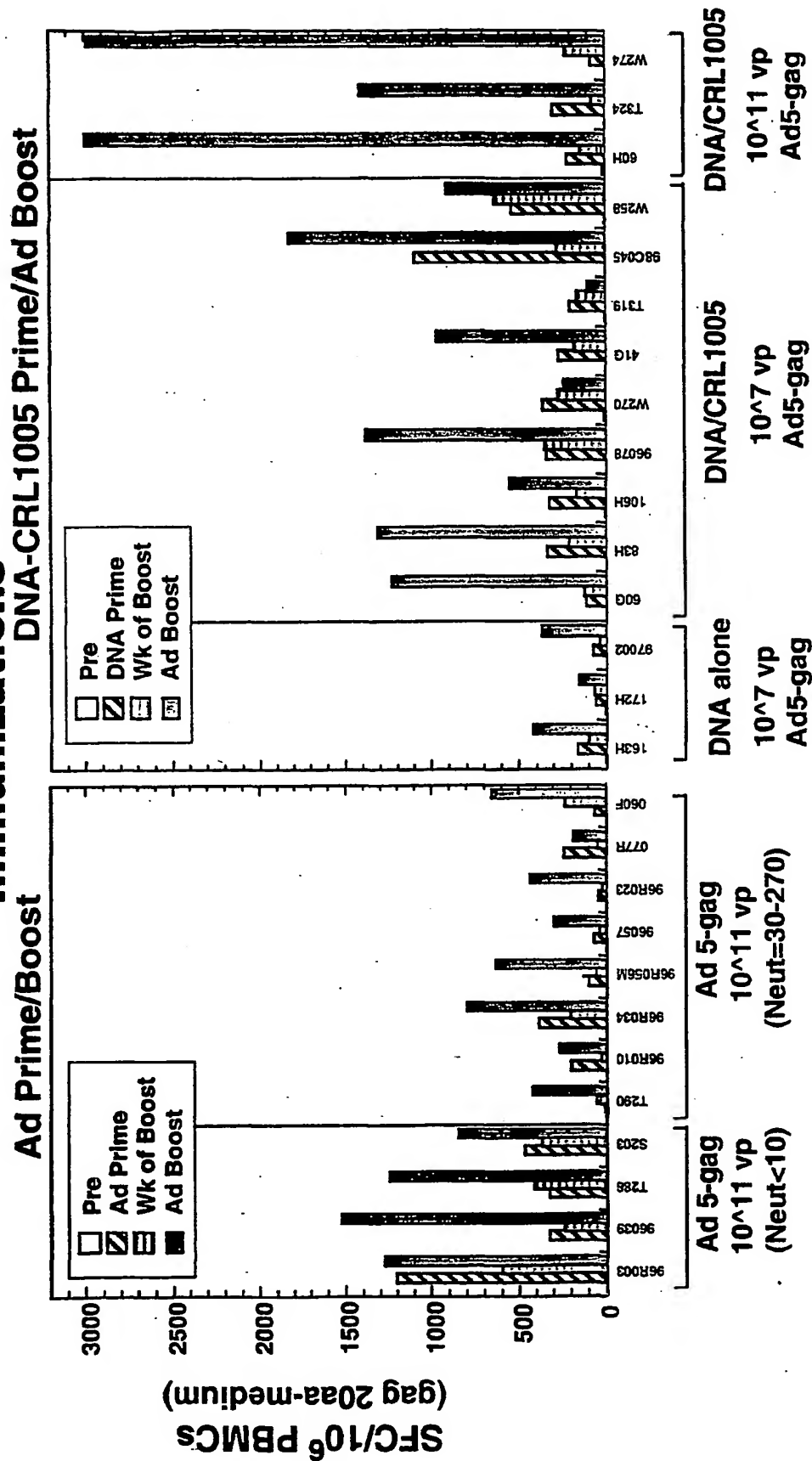


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC
 CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCCAGCA GGCTGCTGCT
 GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG
 GAGAAGGCCT TCTCCCTTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG
 CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT
 GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCATCCC TGTGGGGGAA
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTT
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC
 CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC
 AGGGTGTGCT CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC
 ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC
 CACAAGGGCA GGCCTGGCAA CTTCTTCCAG TCCAGGCCCTG AGCCACAGC CCCTCCCAGG
 GAGTCTTCA GGTTTGGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCAG
 ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC
 CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG
 GAGCTGAACA AGAGGACCCA GGAATTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT
 GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG
 CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCTCCAT CAACAATGAG
 ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC
 AAGAAGCACC AGAAGGAGCC CCCCTTCCCTG TGGATGGGCT ATGAGCTGCA CCCCAGACAAG
 TGGACTGTGC AGCCCATTTG GCTGCCTGAG AAGGACTCCT GGAAGTGTGA TGACATCCAG
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG
GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG
GAGACCTGGT GGA CTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTGTGAAC
ACCCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTGT GGGGGCTGAG
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCTGTGG TGGCTAAGGA GATTGTGGCC
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCTT
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
TCCAAC TTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG
GCTGTGTTCA TCCACA ACTT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCTGTG GAAGGGCCCT
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACTC TGACATCAAG
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA
SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys
 Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp
 Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
 Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln
 Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln
 Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser
 Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser
 Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn
 Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu
 Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala
 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
 Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met
 Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly
 Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp
 Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn
 Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu
 Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu
 Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile
 Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys
 Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr
 Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu
 Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr
 Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr
 Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met
 Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln
 Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr
 Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp
 Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp
SEQ ID NO: 39

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WO 02/22080 A3

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- (26) Publication Language: **English**
- (30) Priority Data:
60/233,180 15 September 2000 (15.09.2000) **US**
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- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **EMINI, Emilio, A.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **YOUIL, Rima** [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **BETT, Andrew, J.** [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CHEN, Ling** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **KASLOW, David, C.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SHIVER, John, W.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **TONER, Timothy, J.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CASIMIRO, Daniel, R.** [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.**
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- Published:**
— with international search report
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS**

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/22080 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
X --- Y	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
X.P	US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X --- Y	US 5,643,579 A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 ----- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C.



See patent family annex.

Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

Box PCT

Washington, D.C. 20231

Facsimile No. (703)305-3230

Date of mailing of the international search report

13 MAR 2002

Authorized officer

Ulrike Winkler, Ph.D.

Telephone No. 703-308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 31
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
This claim could not be searched because applicant did not provide a CRF.
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

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		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .
38	86e, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .
39	86f, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

REVISED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
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(10) International Publication Number
WO 02/022080 A3

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60/233,180 15 September 2000 (15.09.2000) US
- (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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see PCT Gazette No. 30/2002 of 25 July 2002, Section II
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/022080 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 4, 5, 13-17, 29-32, 34, 35, 37 1-3, 8-11, 18
X — Y	US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8.	4, 5, 13-17, 29-32, 34, 35, 37 1, 9, 18
X,P	US 6,287,571 <i>B1</i> (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1-3, 8, 9-11, 18
X — Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	4, 5, 13-17, 29-32, 34, 35, 37 1-3, 9-11, 13-18
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Z" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Gag protein (SEQ ID NO: 29)</u> inserted in the <u>parallel orientation of E1</u> . In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1 and ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Gag protein (SEQ ID NO: 29)</u> .
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV protein inserted in the antiparallel orientation of E1</u> .
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an <u>HIV Gag protein</u> .
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to <u>HIV Gag protein with the recombinant adenoviral particle</u> .
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to <u>HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine</u> .
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the <u>parallel orientation of E1</u> .
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the <u>parallel orientation of E1</u> .
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the <u>parallel orientation of E1</u> .
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the <u>antiparallel orientation of E1</u> .
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the <u>antiparallel orientation of E1</u> .
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the <u>antiparallel orientation of E1</u> .
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u>

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		and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .
38	86e, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .
39	86f, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

CORRECTED VERSION

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[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

WO 02/022080 A3

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S.
provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2
(serial number unassigned), filed September 15, 2000, March 27, 2001, and
September 7, 2001, respectively.

10

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first
generation adenovirus vaccines found to exhibit enhanced growth properties and
20 greater cellular-mediated immunity as compared to other replication-deficient vectors.
The invention also relates to the associated first generation adenoviral vectors
described herein, which, through the incorporation of additional 5' adenovirus
sequence, enhance large scale production efficiency of the recombinant, replication-
defective adenovirus described herein. Another aspect of the instant invention is the
25 surprising discovery that the intron A portion of the human cytomegalovirus (hCMV)
promoter constitutes a region of instability in adenoviral vector constructs. Removal
of this region from adenoviral expression constructs results in greatly improved vector
stability. Therefore, improved vectors expressing a transgene under the control of an
intron A-deleted CMV promoter constitute a further aspect of this invention. These
30 adenoviral vectors are useful for generating recombinant adenovirus vaccines against
human immunodeficiency virus (HIV). In particular, the first generation adenovirus
vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-
1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide
pharmaceutical products, and biologically active modifications thereof. Host
35 administration of the recombinant, replication-deficient adenovirus vaccines described
herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5 The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

10 The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus
15 to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes
20 while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where
25 the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus
30 (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to
35 day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; see, e.g., Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication-defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'-region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in
15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use
20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene

10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral

15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested

20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6[®] cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material

25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual

30 an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,

35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5 The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not
10 limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen
15 with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of
20 such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

 The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be
25 ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)
30 within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second
35 harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair
20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a
25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV
30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a
35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to — highly active antiretroviral therapy —.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by
20 activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a
30 measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along
35 with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression
 5 cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1
 10 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has
 15 been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

20 "MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

25 "MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-
 30 BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*III site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a
 35 plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises
10 codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns
15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as
20 "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone
35 in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5 Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20 Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5 Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed
10 herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences
15 through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate
20 consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding
25 sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as
30 underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino
acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174
35 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5 Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10 Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15 Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20 Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25 Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30 Figure 31 shows the intracellular γ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- γ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ γ IFN+ and CD4+ γ IFN+, respectively.

35 Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6[®] cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention
5 should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular
10 immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human
15 CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as
20 described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrrl isolate wherein the codons are optimized for expression in a
25 mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef
30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for
35 modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon
5 optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with
5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral
10 composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a
15 combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

20 Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.
25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino
30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most
35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells
5 for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully
10 transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of
15 this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

20 Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient
25 to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed
30 *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin
35 resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag) were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene
20 product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8
5 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

10

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVII_{NS}HIVgag was used as the starting material to amplify the hCMV promoter. PVI_{NS}HIVgag is a plasmid comprising the CMV immediate-early
15 (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of
20 the hCMV promoter and a 3' primer (designed to contain the *Bgl*III recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *Bgl*III. This fragment was then cloned back into the original
25 GMP grade pVI_{NS}HIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *Bgl*III digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pVI_{NS}HIVgag vector backbone. This vector is designated pVII_{NS}CMV(no intron).

30 The FLgag gene was excised from pVI_{NS}HIVgag using *Bgl*III digestion and the 1,526 bp gene was gel purified and cloned into pVI_{NS}CMV(no intron) at the *Bgl*III site. Colonies were screened using *Sma*I restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pVI_{NS}CMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence
35 integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	$\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$
HIVFL-gagPR9901 ^a	10.8
PVJns-hCMV-FLgag-bGHpA ^b	16.6
pV1Jns-hCMV-FLgag-SPA ^{bc}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

5 ^b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

10

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above

15 possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No.

PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.

Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.

Application Serial No. 60/148,981, filed August 13, 1999, all three applications which

20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20 μg and 200 μg .

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA ^a Promoter/terminator	Dose, ug ^b	Anti-p24 Titers (3 Wk PD1) ^c			SFC/10 ⁶ Cells (4 Wk PD1) ^d		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV- FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV- FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

^ain PBS^bi.m. Injections into both quads, 50 µL per quad^cn=10; GMT, geometric mean titer; SE, standard. error^dn=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

Construction of the Modified Shuttle Vector -"MRKpdeIE1 Shuttle"

- The modifications to the original Ad5 shuttle vector (pdeIE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:
- (1) The left ITR region was extended to include the *Pac*1 site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
 - (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdeIE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdeIE1 shuttle) with *PacI* and *BstZ1101* and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *ClaI* linearized pAdHVO (E3- adenovector) or *ClaI* linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *ClaI*, *BamHI*, *Xho I*, *EcoRV*, *HindIII*, *Sal I*, and *Bgl II* sites. This MCS was replaced with a new MCS containing *Not I*, *Cla I*, *EcoRV* and *Asc I* sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and then labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene –
“MRKpdelE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeIE1 shuttle vector.

EXAMPLE 9

Construction of the MRK FG-Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *PacI*. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *ClaI* overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH₂O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bsf*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1 gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac1* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6[®] cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6[®] cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [³³P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac1/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5 Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10 Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10 ⁶ cells/ml), Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁶ vp/ml culture	Titer 10 ⁶ vp/cell	OPA 10 ⁶ TCID ₅₀ /ml	Ratio AEX/QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 83%	0.56, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 84%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.95, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 59%	50	52	6.2	4.7	1.70	81	170	
P8	1.03, 94%	0.85, 64%	47.5	54	9.0	6.7	1.10	82	310	
P9	0.69, 95%	0.93, 73%	47.5	56	4.4	4.9	1.03	43	178	3.12 2.84
P10	1.09, 91%	1.06, 66%	47.5	58	3.0	2.8	1.16	26	100	2.70 2.60
P11	1.18, 88%	0.88, 63%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.88 2.60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 82%	0.88, 67%	46	53	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10 ⁶ cells/ml), Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁶ vp/ml culture	Titer 10 ⁶ vp/cell	OPA 10 ⁶ TCID ₅₀ /ml	Ratio AEX/QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 78%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.82, 83%	1.18, 77%	47	49	4.3	4.7	1.24	35	170	
P6	1.55, 86%	1.28, 76%	49.5	50	1.2	0.8	0.58	21	30	
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.18	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 63%	48	56	2.1	2.1	0.47	45	75	3.12 2.84
P9	1.20, 89%	1.26, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.53, 86%	47	50	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.23, 83%	48	47	2.7	2.6	0.41	66	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.8	7.4	0.48	123	280	3.18 3.18
P13	1.96, 95%	1.14, 85%	48.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 88%	48.5	47	9.4	8.7			350	3.12 2.91
P15	0.67, 99%	0.97, 83%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

MRKAd5gag(E3-)

	Xv (10 ⁶ cells/ml)	Viability (%)	Harvest Time h.p.i.	Cell Passage Number	Tit _r 10 ⁶ vp/ml culture	Tit _r 10 ⁶ vp/cell	QPA 10 ⁶ TCID ₅₀ /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 64%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	8.1	3.2	0.66	47	115	3.12
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.67	32	85	2.84
P10	0.99, 82%	0.60, 62%	48.5	60	3.2	3.2	0.68	47	115	2.70
P11	1.07, 96%	0.88, 70%	48.5	47	5.9	5.5	0.68	87	200	2.60
P12	0.80, 81%	0.57, 59%	50	49	6.1	6.4	0.72	71	230	2.85
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.18
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.28
P15	0.87, 89%	0.84, 66%	49	49	4.8	5.5			196	3.27
										2.78
										2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10^7 and 10^9 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors ^a	$\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^e	0.42

^a $A_{260\text{nm}}$ absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

^d Research Ad5FLgag lot# 6399

^e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	^a MRKAd5gag	10 ⁷	25600	5877	4780
2	"	10 ⁹	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10 ⁷	7352	2077	1620
4	"	10 ⁹	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10 ⁷	12800	9905	236
6	"	10 ⁹	310419	99181	75165
7	^b mCMV FL-gag bGHpA [E3+] →	10 ⁷	44572	23504	15389
8	"	10 ⁹	941014	239068	190836
9	^c hCMV FL-gag bGHpA [E3-] ←	10 ⁷	3676	934	745
10	"	10 ⁹	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10 ⁶	528	262	175
12	"	10 ⁷	14703	5274	3882
13	"	10 ⁸	58813	14942	11915
14	"	10 ⁹	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10 ⁶	230	82	61
16	"	10 ⁷	4222	3405	1138
17	"	10 ⁸	19401	3939	3274
18	"	10 ⁹	89144	25187	19639
19	Naïve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag

Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10¹¹ vp and 10⁹ vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood assmumarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after
- 5 CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag ^P , 10 ¹¹ vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10 ⁹ vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag ^P , Clinical Lot, 10 ¹¹ vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10 ⁹ vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
^P MRKAd5gag (hCMV, bGHpA, E3+)								
^P original Ad5gag vector (hCMV/Intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media ^a	Gag H ^b	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag 10 ⁹ vp	97ND10	8	89	0	395	0	1058	0	1174	3	775	4	1074
		97ND10(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2676			0	1656	0	1278
2	MRKAd5gag 10 ⁹ vp	97N120	5	275	1	249	4	141	4	119	9	206	4	219
		97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot 10 ⁹ vp	97X001	0	281	1	485	0	817	0	1220b	1	894	0	1858
		97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot 10 ⁹ vp	97ND20	3	30	1	101	0	66	0	36	0	26	0	41
		97ND20(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Native	96RD41	8	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16	20	14	19	15	10	15	24	9

Based on either 4x10⁶ or 2x10⁶ cells per well (depending on spot density)

ND, not determined

^aMock or no peptide control

^bPool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10⁹ vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

15

EXAMPLE 17

CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

20

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

```

AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCACTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

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GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
 CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
 5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
 AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
 CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
 GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
 10 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
 CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
 15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
 20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
 GGGGCTGAGA CTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
 25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
 ATCAGGAAGG TGCTGTTCTT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
 CACTCCAACT GGAGGGCTAT GGCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC
 30 TGCTCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGA GTCCATGAAC
 35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to
 deletion of the portion of the wild type sequence encoding the protease activity, a
 30 combination of active site residue mutations are introduced which are deleterious to
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein
 the construct is devoid of DNA sequences encoding any PR activity, as well as
 containing a mutation(s) which at least partially, and preferably substantially,
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IAPol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IAPol":

```

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCCTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGAAGGAGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTTGT
GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGAAGTACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCGAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGTGGC
35 ATCAGGAAGG TGCTGTTCCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

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ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 TGCTCCCTCG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTAT CCACAATTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 10 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGG ACTCCAGGAA CCCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAATCTT
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
 NO:3).

15 In order to produce the IA-pol-based adenoviral vaccines of the present
 invention, inactivation of the enzymatic functions was achieved by replacing a total of
 nine active site residues from the enzyme subunits with alanine side-chains. As
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*
 1989; 86: 4803-4807). Three additional mutations were introduced at Asp445,
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this
 IA Pol construct), with each residue being substituted for an Ala residue, respectively
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase
 function was abolished through three mutations at Asp626, Asp678 and Glu714.
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based
 adenoviral HIV vaccine of the present invention, either when administered alone or in
 a combined modality regime and/or a prime-boost regimen. For example, it may be
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,
 RNase-H, and integrase coding regions while still abolishing these enzymatic
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide
 such as is found in highly expressed mammalian proteins such as immunoglobulin
 leader peptides. Any functional leader peptide may be tested for efficacy. However,
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,
 preferably a leader peptide from human tPA. In other words, a codon optimized
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide
 at the amino terminal portion of the protein, which may effect cellular trafficking and
 hence, immunogenicity of the expressed protein within the host cell. As noted in
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCAT TGTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCA CACCAATGAT GTGAAGCAGC TGA CTGAGGCTG
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCATT GTGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 15 GGAGGTGAAC ATTGTGACTG ACTCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGACAATG GCTCCAATT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACCTCAGG GTGTACTACA GGGACTCCAG
 30 GAACCCCTG TGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)
 which comprises a leader peptide at the amino terminal portion of the protein, which
 may effect cellular trafficking and hence, immunogenicity of the expressed protein
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,
 any such leader peptide-based HIV-1 pol mutant construct may include but is not
 limited to a mutated DNA molecule which effectively alters the catalytic activity of
 the RT, RNase and/or IN region of the expressed protein, resulting in at least
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at
 least one point mutation which alters the active site and catalytic activity within the
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed

5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open

10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA

15 GCTGAAGCCT GGCATGGATG GCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT

20 GGGGGATGCC TACTTCTCTG TGCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCTTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCCTG AGAAGGACTC
CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA

30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
GCAGGGCCAG GGCCAGTGGG CCTACCAAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGGAGGC
TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT
GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT

35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

- 5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfr1
 10 nef gene is disclosed herein as SEQ ID NO:9, as shown herein:
 GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
 15 GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
 TGTCCTACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
 ACACCCCCGG CCCCGGCATC AGGTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACATGC CTGCTGCACC
 20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
 CCAAGCTGGC CTTCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
 AAAGCCCGGG C (SEQ ID NO:9).

- Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),
 Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);
 25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),
 Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian
 (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby
 incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.
 codon optimized nucleotides comprising the open reading frame of HIV-Nef.

- 30 The open reading frame for SEQ ID NO:9 above comprises an initiating
 methionine-residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides
 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid
 HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine
 vector. The 216 amino acid HIV-1 Nef (jfr1) protein is disclosed herein as SEQ ID
 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions
 have been elucidated, it has become clear that correct trafficking of Nef to the inner
 plasma membrane promotes viral replication by altering the host intracellular
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the
 20 infectivity of progeny viral particles. In one aspect of the invention regarding
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the
 adenovirus vector of the present invention is modified to contain a nucleotide
 sequence which encodes a heterologous leader peptide such that the amino terminal
 region of the expressed protein will contain the leader peptide. The diversity of
 25 function that typifies eukaryotic cells depends upon the structural differentiation of
 their membrane boundaries. To generate and maintain these structures, proteins must
 be transported from their site of synthesis in the endoplasmic reticulum to
 predetermined destinations throughout the cell. This requires that the trafficking
 proteins display sorting signals that are recognized by the molecular machinery
 30 responsible for route selection located at the access points to the main trafficking
 pathways. Sorting decisions for most proteins need to be made only once as they
 traverse their biosynthetic pathways since their final destination, the cellular location
 at which they perform their function, becomes their permanent residence.
 Maintenance of intracellular integrity depends in part on the selective sorting and
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

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CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCAGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTGC ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfrl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCC GGCTGG TCCACCGTGA
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCTCCA
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
 ACACCCCCGG CCCC GGCCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCACC
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
 AAAGCCCCGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA
 30 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue
 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
 TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCGAGGAG GACGAGGAGG TGGGCTTCCC
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
 CTTCTTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGA
 GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCTG ACTCCAAGCT
 GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCCC
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,

regardless of codon usage, which expresses a wild type or modified Nef protein as

35 described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BglII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)ClaI (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the PacI site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with Bgl II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using

5 restriction enzymes *DraIII/NotI*. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *PacI* and *Bst1107 I* (or its isoschizomer, *BstZ107 I*) and then co-transformed into *E. coli* strain BJ5183 with

10 linearized (*ClaI* digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)ClaI. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA

15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *PacI* (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate co-

25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *PacI* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing

30 recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the MRKpdeIE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac*1 (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *Pac1* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at $\leq -60^{\circ}\text{C}$. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

10 The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent

15 the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR

20 product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4

25 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel

30 orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length

35 IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

EXAMPLE 22

5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla*I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated).
25 Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca*I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac*I and *Bst*Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial
30 homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were collected from all the animals for RT ELISA and IFN γ ELISPOT analyses, respectively. For all rodent immunizations, the Ad5 vectors were
5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following
10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were
15 collected from all the animals for RT ELISA and IFN γ ELISPOT analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁹ vp and 10¹¹ vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either
20 10⁹ vp and 10¹¹ vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0)
25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μ L of 1 μ g/mL HIV-1 RT protein
30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 μ L of 1 μ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200 μ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was
35 performed followed by 4-fold serial dilution. 100- μ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by
5 adding 100 μ L of 0.5M H₂SO₄ per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELISpot assays - The enzyme-linked
10 immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF γ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5×10^6 /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL
15 streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μ L/well of either 5 μ g/mL purified rat anti-mouse IFN- γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or
20 15 ug/mL mouse anti-human IFN- γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μ L of cell samples ($4-5 \times 10^5$ cells per well) and 50 μ L of the
25 antigen solution were added. To the control well, 50 μ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790)
30 or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap
35 by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-γ goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁷ vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers ^a			SFC/10 ⁶ cells ^b		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10 ⁷ vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10 ⁹ vp	2 1	1838400 ^b 713155	0 528520	0 303556	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10 ⁷ vp	2 1	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 ⁹ vp	2 1	1838400 ^b 1241675 ^b	0 396725	0 300681	1(1) 0(0)	180(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNear or at the upper limit of the serial dilution; hence, could be greater than this value^cNo. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers ^a			SFC/10 ⁶ cells ^b		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10 ⁷ vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10 ⁹ vp	2 1	174 132	70 42	50 32	0(0) 1(1)	81(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10 ⁷ vp	2 1	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 ⁹ vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10 ⁷ vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10 ⁹ vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52	21(2)	18(6)	25(3)

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNo. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IAPol(E3+) 10^{11} vp	99C100	1	0	0	1	38	31	0	52	146	0	49	715
	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IAPol(E3+) 10^9 vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	192	4	38	158	5	38	108
	99C201	8	5	21	8	62	62	0	18	32	1	14	65
MRKAd5hCMV-IAPol(E3-) 10^{11} vp	99D239	5	2	2	20	82	172	1	68	114	9	21	40
	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	484	0	14	238	1	24	284
MRKAd5hCMV-IAPol(E3-) 10^9 vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	178
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Ndve	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined
Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mIU/mL				
Vaccine/Monkey T og	T=4	T=7	T=12	T=16
MRKAd5hCMV-IAPol(E3+), 10^{11} vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IAPol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IAPol(E3-), 10^{11} vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IAPol(E3-), 10^9 vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 11 vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 9 vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	8	1148	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 11 vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ⁶ 9 vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naive	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

- Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects
- PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10^6 cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10^{10} vp/ml culture	Titer 10^4 vp/cell	Amplification Ratio	Triton Lysis Titer 10^{10} vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10^6 cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10^{10} vp/ml culture	Titer 10^4 vp/cell	Amplification Ratio	Triton Lysis Titer 10^{10} vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of
- 20 MRKAd5gag. PER.C6[®] cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral
- 25 particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

5 *Comparison of hCMV- and mCMV-FL-nef* - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the
10 four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

15

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 ⁶ cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10 ⁶ vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef (MRKAd5nef)	Pool	1.11, 91%		60	1.5	1.4	50	2.8
	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

20

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate,
25 no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x10⁶ cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10⁶ cells/ml. The cells were infected with uncloned
30 MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1×10^{13} vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1×10^{11} IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10^7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10^7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, $CD4^+$ -biased or $CD8^+$ -biased, and (b) boosting with the MRKAd5gag
30 construct produced in all cases a strongly $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific $CD8^+$ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag

Grp	Priming	Boost	Monkey	T=0		T=4		T=8		T=10		T=17		T=24		T=28		T=30	
				Medium	ppg H	Medium	ppg H	Medium	ppg H	Medium	ppg H	Medium	ppg H	Medium	ppg H	Medium	ppg H	Medium	ppg H
1	T=0, 4, 8 wks DNA5 mgs PBS (0101)	T=28 wks MRKAd5gag(E3+) 10 ⁷ vp	CB5H	NA	3	35	15	71	4	224	8	115	6	05	19	856	0	318	
			CC6X	0	0	15	0	48	0	78	0	35	3	1705	1	755			
			AW3G	5	11	0	36	3	51	3	48	2	89	8	66	10	888	0	395
2	DNA5 mgs + CRL1005/45mgs	MRKAd5gag(E3+) 10 ⁷ vp	CC1C	0	4	1	60	0	111	5	270	4	260	8	232	3	959	19	1345
			CC1K	4	0	1	101	0	264	0	791	5	452	0	321	0	1918	1	1099
			AW3P	9	8	1	10	4	71	4	184	8	104	5	85	11	836	8	241
			CB5F	NA	0	31	0	268	0	530	19	374	9	251	8	1734	20	1734	
			AW8B	8	12	4	38	1	119	0	439	0	425	0	310	4	1229	6	1364
3	DNA5 mgs + CRL1005/7.5 mgs + 0.8 mM BAK	MRKAd5gag(E3+) 10 ⁷ vp	AW20	10	4	1	59	5	264	18	425	6	105	9	205	18	565	9	404
			CA4R	1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
			CB5B	8	6	0	6	3	119	6	274	6	282	1	208	0	636	1	828
			CB5W	4	3	0	26	1	91	0	139	0	164	1	62	5	643	1	349
			CB7D	1	0	0	138	0	318	1	609	5	625	1	769	0	2278	4	1831
4	none	None	88D201	3	0	0	0	1	0	0	0	0	1	2	3	0	0	0	

NA, not available

NA: not available

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused
5 directly to the open reading frame of the IAPol gene (consisting of RT, RNaseH and
integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not
include the protease gene and the frameshift sequence, it encodes a single polypeptide
of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID
NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last
non-stop codon was ligated via PCR to a fragment that extends from the start codon
of the IAPol to a unique BamHI site. This fragment was digested with BstEII and
BamHI. Construction of gag-IAPol fusion was achieved via three-fragment ligation
involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII
fragment of the V1R-gagpol containing the entire ORF of gag-IAPol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

20 Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral
particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag;
(2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of
MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and
4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-
gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein
30 sequence of each antigen. The results (Table 25) are expressed as the number of spot-
forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that
respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene
constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels
35 of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can
be mixed as a multi-cocktail formulation capable of eliciting very broad T cell
responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

5 **Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10 ¹⁰ vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10 ⁸ vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10 ¹⁰ vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10 ⁸ vp	AW38	1	-	19	30	-
		CC8K	0	-	50	895	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10 ¹⁰ vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10 ⁸ vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ¹⁰ vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ⁸ vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10 ¹⁰ vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10 ⁸ vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10⁶ PBMC.

WHAT IS CLAIMED IS

1. A recombinant adenoviral vaccine vector at least partially deleted in
5 E1 and devoid of E1 activity, comprising:
- a) an adenovirus *cis*-acting packaging region corresponding to from
about base pair 1 to between from about base pair 400 to about
base pair 458 of a wildtype adenovirus genome; and
 - b) a gene encoding an HIV protein or immunologically relevant
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region
corresponding to from about base pair 1 to about base pair 450 of a wildtype
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides
15 corresponding to between from about base pair 3511 to about 3524 to about base pair
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a
10 physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,
15 replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6[®] cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
20 claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell
15 line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6[®] cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

49. An adenoviral vector in accordance with claim 9 wherein the gene
20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
- ii) a heterologous promoter operatively linked to i); and
- iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6[®] cell.

15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

20 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10 75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

20

80. A method according to claim 79 wherein the cell is a PER.C6[®] cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;

and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with
claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to a single promoter; and the encoding nucleic acid sequences
operatively linked by an internal ribosome entry sequence ("IRES").

Sequence of the open reading frame for FL-qsg (human codon optimized)

atggtgtctagaggctctctgctgtcttgggttgagctggacaagtgaggagaagatcaggctgaggccctgggg
caagaagaagtacaagctaaagcacattgtgtggccctccaggagctggagagggttctgtgaaccttggc
ctgtgtggagacctctgaggggtgtagggcagatcttgggccaactccagccctccctgcaaaccagggtctgagg
agctgagggtccctgtacaacacagtggttaccctgtactgtgtgaccagaaagattgattgtaaggacaccaag
gaggccctgggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcagggtctgtcttggc
acagggaactccagccagggtgtccagaactccccattgtgcagaacctccaggggccagatggtgcaaccag
gcatctccccccggaaccttgaaatgctgggtgaagggtgtggaggagaaggcccttcccttgagggtgaccc
catgttctctgcccgtctgagggtgtccacccccaggacctgaacacattgtcgaacacagtggggggccatc
aggctggccatgcatgtctgaaggagaccatcaatgagagggtctgtgagtgaggacaggctgcactctgtgc
acgtctggccccattgtccccggccagatgaggggagccaggggctctgacattgtgtggcaccctccacct
ccaggagcagattggctggga'gaccaacaaccccccatccctgtgggggaaatctacaagggtggatcat
ctggggctgaacaagattgtgaggatgtactccccaccttccatcttggacatcaggcaggggccccaaggag
ccctcagggactatgtggacagggttctacaagaccttgagggttgagcaggccctccaggagggtgaagaact
ggatgacagagacctgtctgggtgcagaatgcaaccttgactgcaagaccatctgaaaggccctgggcccctg
ctgccaaccttggaggagatgtatgacagcttggcagggggtggggggcccttggtcaaggccagggtgtctg
gtctagggccatgtcccaggtagcaaacctccgccacatcatgaltgcagaggggcaacttcagggaaccagag
gaagacagtgaagtgcttcaactgtggcaagggtgggccacattgccaagaactgtlagggcccccaaggaga
agggctgtctggaaggtgtggcaaggaggggccaccagatgaaggactgcaatgagaggcaggccaactctg
ggcaaaactctggccctcccaaggaggcagggtggcaactctccagtgccaggcttgagcccaagcaccct
ccaggaggagcttcaggttggggaggaggaagaccacccccagcagaaggcaggagccattgacaagg
agctgtaacccccctggccctccctgagggtccctgttggcaacgacccctccctccaglaaaaataagccccggca
gat (SEQ ID NO: 29)

Figure 2

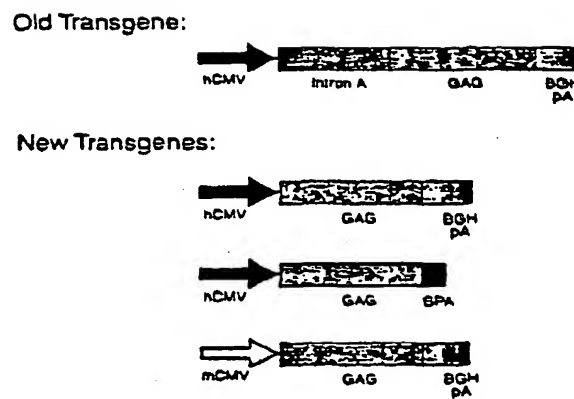


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

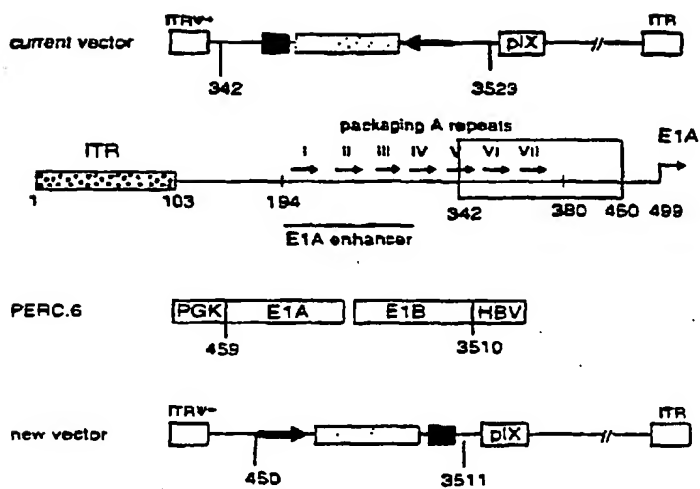


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

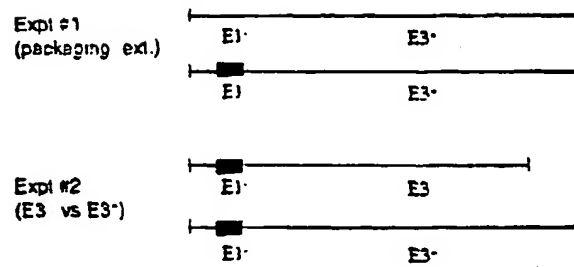


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

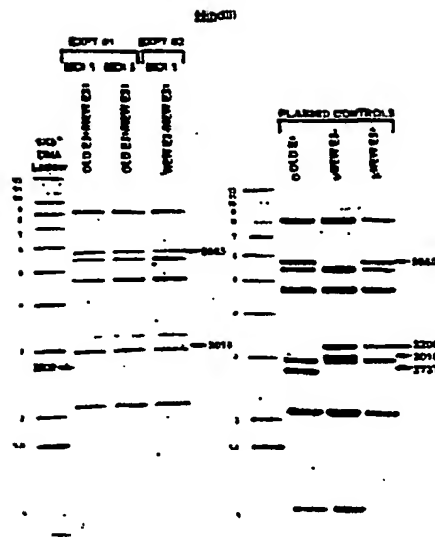


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

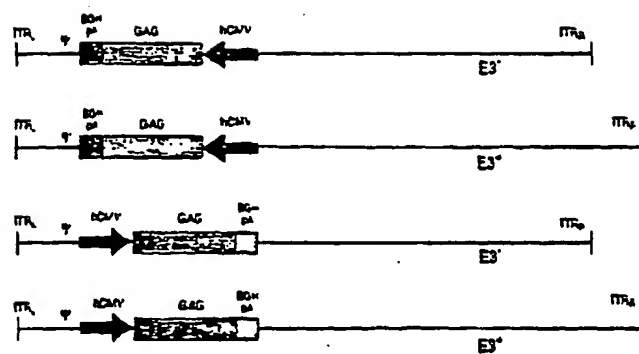


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3⁻ and E3⁺ backbones were constructed.

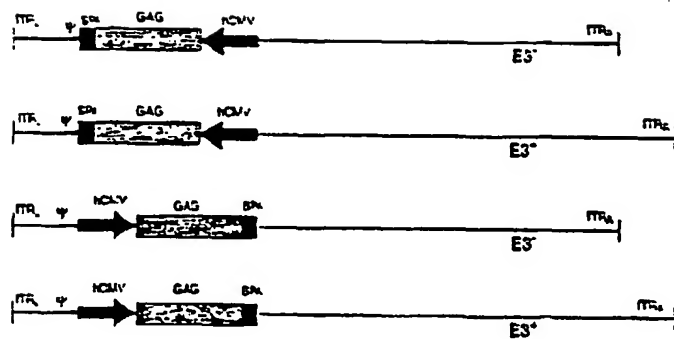


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3⁻ and E3⁺ backbones were constructed.

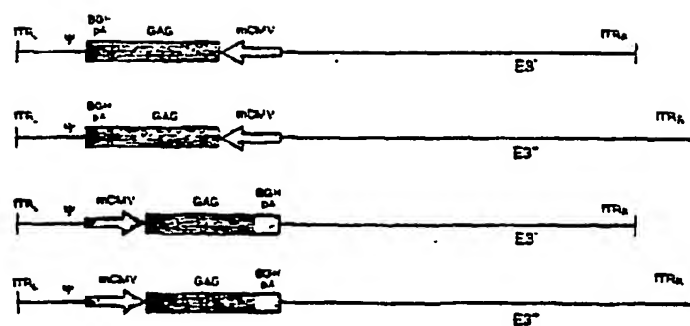


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

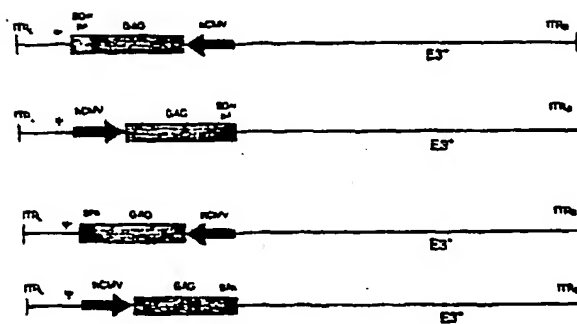


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

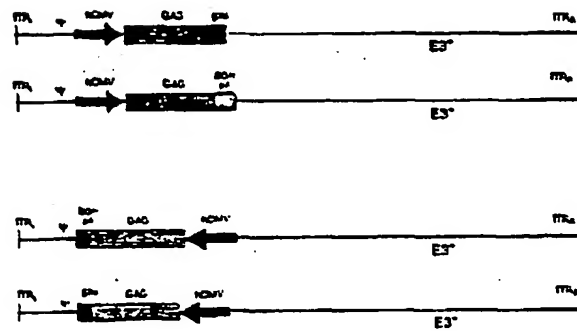


Figure 8B: Effect of polyadenylation signal

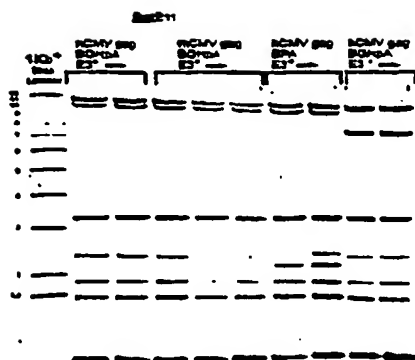


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.

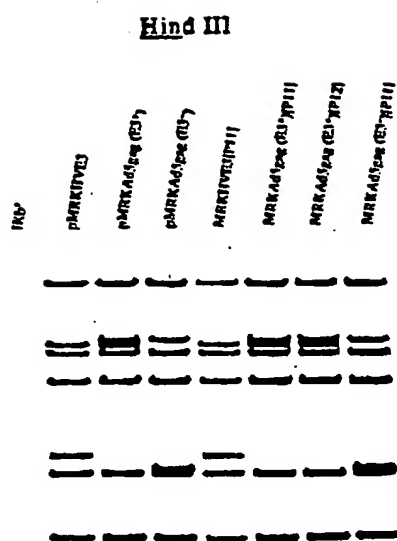


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

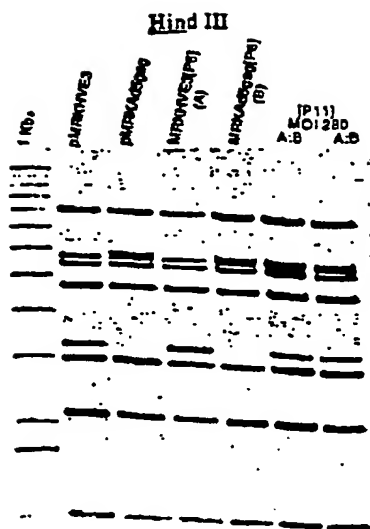


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

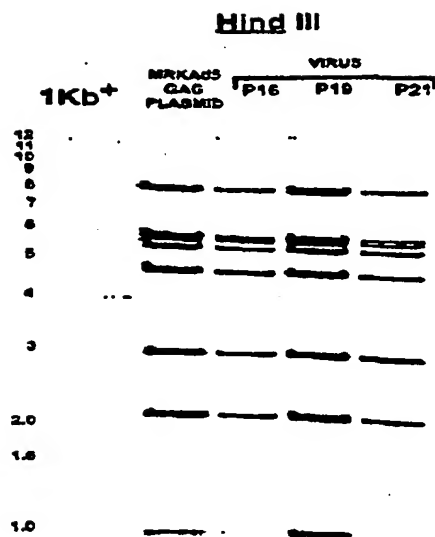
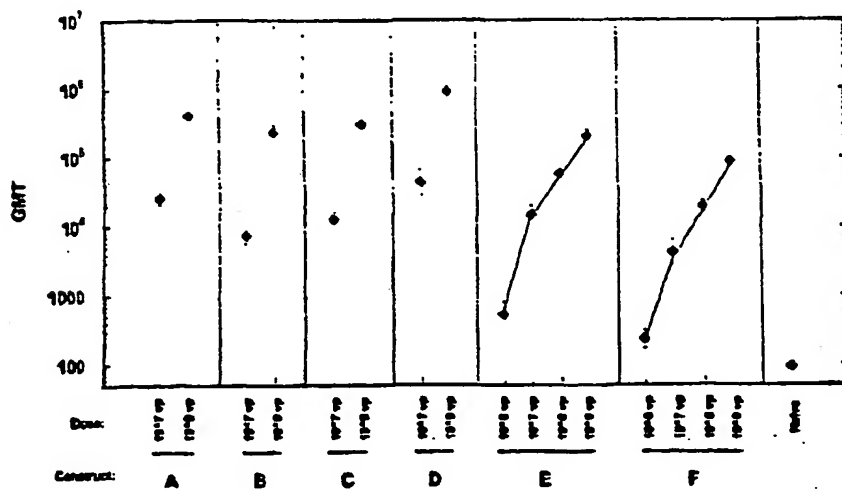


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

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Figure 13. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3⁺ bCMV-FLgag-bGHpA; (C) MRKAd5 E3⁺ bCMV-FLgag-SPA; (D) MRKAd5 E3⁺ mCMV-FLgag-bGHpA; (E) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



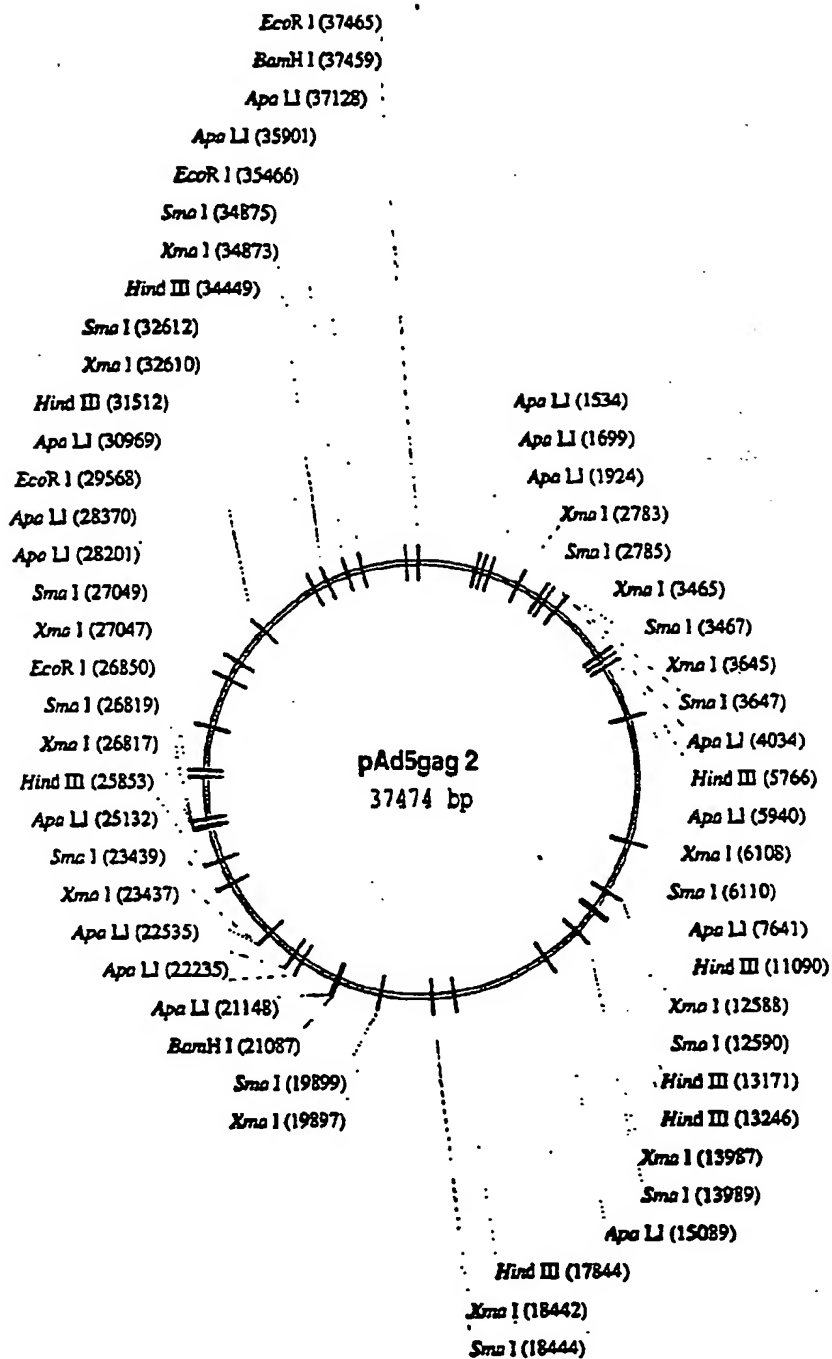


Figure 14

	Field Accession Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522
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[illegible]

[illegible]

20/144

[illegible]

Figure 15D

pMRKad5qng MER6R2

6501 GCGTCACGCA GAGAGAGGCG GTACAGAGTCTT TCACTAGATCT GACCTGTAAC TCACTAGATCT GAGAGAGTCTT TCCCTGATGA
 CCGAGTACGCT GCTTCTCTCG CATCTCTCAGC GGTCTCTCAGC ACTGATGATG GGTCTCTCAGC CCGAGTACGCT CCGAGTACGCT
 6601 TGTCTACTCTT ATCTCTCTCG TTTTCTCTCG ATCTCTCTCG TTTTCTCTCG ATCTCTCTCG TTTTCTCTCG TTTTCTCTCG
 ACAGTATGAA TAGAGAGGCG AAGAGAGGCG TGTCTCTCTG CACTCTCTCT TGTCTCTCTG CACTCTCTCT TGTCTCTCTG
 6701 CCGAGTACGCA GAGCTCTGCA TGTCTCTCTG GTCTCTCTCT GTCTCTCTCT GTCTCTCTCT GTCTCTCTCT GTCTCTCTCT
 GCTTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT
 6801 GAGTCTCTCG TGTCTCTCTG GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT
 CTCACACGCG ACTCTCTCTT CACAGAGGAG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG
 6901 CCGTCTCTCT TTTCTCTCTG GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT
 GCGAGAGGAA AAGCTCTCTG CCGTCTCTCT CCGTCTCTCT CCGTCTCTCT CCGTCTCTCT CCGTCTCTCT CCGTCTCTCT
 7001 TCCCTCTCTC TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG
 AGGCTCTCTG AGGCTCTCTG AGGCTCTCTG AGGCTCTCTG AGGCTCTCTG AGGCTCTCTG AGGCTCTCTG AGGCTCTCTG
 7101 GCGTCTCTCT TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG
 CCGTCTCTCT ACTCTCTCTG CAGTCTCTCT CAGTCTCTCT CAGTCTCTCT CAGTCTCTCT CAGTCTCTCT CAGTCTCTCT
 7201 GCGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT
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 CCGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT GAGTCTCTCT

Figure 15E
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[illegible]

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pMHRAd5gag MER682

11301	TCGATTTGAT AACATCTG CAGATATATG TTTTATATGA GTTATATTTT ATCTTGTCTT TTTTATATAT CCGATCAAC TATTCCATGC TTATGCTGTT
11401	ACCTAACTA TTTGTAGGAC GTCTGTATG ACATCTCTCT CTCTATATAC TTTTATATAT TTTTATATAT TTTTATATAT TTTTATATAT
11501	CAGATTTTAC GCGGCAAGA TATATATATG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
11601	ACCTTATGCG ACATCTCTG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
11701	CCCTGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
11801	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
11901	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12001	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12101	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12201	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12301	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12401	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12501	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12601	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12701	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT
12801	CCATGATGCA GCTTGTGCG CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT CCGTATATAT

Figure 15H

[illegible]

26/144

pMIRKAD5qag MEIR6R2

14501	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
14601	AGGCGGACAG ATACGACATG CCAATTTTAA GCGGTATGTA TCTATGATTT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT	GGGATGTTCT
14701	GGGATGTTCT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT	GGGATGTTCT
14801	AGGCGGACAG ATACGACATG CCAATTTTAA GCGGTATGTA TCTATGATTT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT	GGGATGTTCT
14901	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15001	AGGCGGACAG ATACGACATG CCAATTTTAA GCGGTATGTA TCTATGATTT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT TGTATGTTAT	GGGATGTTCT
15101	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15201	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15301	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15401	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15501	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15601	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15701	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15801	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
15901	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT
16001	CTTACGATGTA TCTGTGAGGCT GTTACATTTT CCGCACTTCT GTATGTTGAC GTCTACTTAT GTAGCTTTAA AGATGACATC GATACATGCG GGGGTGTGCT	GGGATGTTCT

Figure 15J

[illegible]

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pMRKAl5qag MRR6B2

17601	EcoRV TGGGACCCAG CAAATATGCG GGTGGGCGCT TCAGTGTGGT CTTGCTGCTT AGTGTATTTA AATATTTTGT TTCCACCTGT AATGACTATG GCAGTAAAGT
17701	AGCGGTGGTC GTTATATCTG AGTGTGCTGC GAGCTAGACT TTGGCTTAAT TTATAAATGC AAGGTGCGAA TTCTTATATC GTTGTGTTCT
17801	CTGGACACGC AGCAGAGGCT AGATGCTGAG GATATATTTG AATATGAGAA ATTATGAGAA AATATGCTTA GATGAGCTGT CTTGTGCTAT
17901	GACCTGTGCG TGGTGTGCGG TCTAGCGACT CCTATTCAAC TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT
18001	GTGGACCTGT CCAACACGCG AGTGCNAT AGATTATCA GTATTTTCTA TCCGCGCTT CCGTTAGAGG AGCTCTACG GGGGCTGGAG ACAGTGTCT
18101	CACCTGGGAC GTTGTGTCGG TCACTGTTTGA TTCTTAATTTGT CATTGTATCT AGTGTGCTTA TCCGCGCTT CCGTTAGAGG AGCTCTACG GGGGCTGGAG
18201	CAGAGGCGCG TGGCGAAGAG CGTTGCGGCT CCGACAGGTA AGATATCTAT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT
18301	GTCTGCGCGC ACCGCTTTTC GCAAGCGCGCG GACTGTGCGT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT TTTTCTTTCT
18401	CTTGCCTGAC ACCGCTTTTC TCGGCGCTAT GGTACTGTGA GTTGTGCTA GTTGTGCTA GTTGTGCTA GTTGTGCTA GTTGTGCTA
18501	GTACGCTGAG AGCGCGCTTC TCGGCGCTAT GGTACTGTGA GTTGTGCTA GTTGTGCTA GTTGTGCTA GTTGTGCTA GTTGTGCTA
18601	AGAAAGCCCA CGGTGCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA
18701	CTTGTGCGCT GCGACCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA
18801	CTTGTGCGCT GCGACCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA
18901	CTTGTGCGCT GCGACCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA
19001	CTTGTGCGCT GCGACCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA
19101	CTTGTGCGCT GCGACCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA
19201	CTTGTGCGCT GCGACCGCGC TACGACACGAC GTGACACGAC AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA AGCTGTGCTA

Figure 15L

PMKRA1594q HER6R2

21001	TTATGTCAT	GGGCGACTC	ACAGAGCTG	CCGAAAGCT	TCCTATATC	ATCTGCTCC	ACGCGTACA	CATGACTTT	GGGTCGATC	CGATGAGTA
21101	ATACAGGTA	CCCGGCTAG	TGCTGTACC	CGTTTTCGA	ATAGATGTA	TTGAGCTGG	TGCGATATC	GTACTGAAA	CTCGACTAG	GTATCTGCT
	GGGCGAGCT	CTTTATGTT	TGTTGAGCT	CTTTGAGCT	GTCTGCTGC	AGCAGTCTA	CTCTGCTGC	ATGCAAGCT	TGATCTGCT	CAAGCTCT
	CGGCTGAGG	GAATATGAA	ACAACTTCA	GAAGCTGAC	CAAGCTGAC	TGCTGCTGC	GTCTGCTGC	TGCTGCTGC	ATCTGCTGC	GTCTGCTGC
21201	TGCGCGGTA	ACGCGGAGC	ATAGAGAGC	AGCGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC
21301	ATCGCGGCT	TGCGGCTAG	TATTTCTTC	TGCGGCTAG	TGCGGCTAG	TGCGGCTAG	TGCGGCTAG	TGCGGCTAG	TGCGGCTAG	TGCGGCTAG
21401	ACGAGAGCT	GGTATAGAA	ACCGCTGAT	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC	ATAGAGAGC
21501	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
21601	ATGTTTACCA	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT
	ATGTTTACCA	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT
21701	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
21801	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
21901	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
22001	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
22101	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
22201	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
22301	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
22401	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC
22501	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC	GTCTGAGCT	CGATAGAGC

Figure 15N

[illegible]

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Figure 1502

pMIRAd5gag HER682

27301 CTTCCCGCC ACTATCGCGA TCATTTTAT CTTACACTTTG AGCTGTATTA GCACTCGGCT GACCGCTACG ACTGATGTTT AGGTGTCGAG GCAGAGCCAG
GAGGCGCGCG TGTATAGGCTT AGTTTATTA GATTATTAAG TGTCTTATTT CTTGAGCCGC CTGATGATTC TTACTTACAA TTACTCTCTC GGTCTCTTTT
TGGCCCTGAA AGACCTGCTC CACTGTCTCC GCACTGATTC CTTTATCTG GACTTCGGTG ATTTTGTCTA CTTTGAATTT CCGGAGATTC ATATCTGAGT
ACGCGGACTT TGTGAGCCAG GTGACAGCGG CGGTGTTTAC GAAATCTCC CTGAGCCGC TGAACGCT GAACTTTAAC GCGCTGCTAG TATAGCTCTT
CGCGGCGCAC GCGGTGCGGC TTACCGGCGA GGAAGATCTT GCGGTGATC TCAATCTGGA GTTTACCGAG CCGCGCTGCG ATGATCTGCG GCTGTGCGC
GCGCGCGCTG CCGGAGCGCG AATGCGGCT CCGCTCTGGA CGGCGATCGG ACTAAGCCTT CAATGCGCTC GCGGCGGAGG ATCAACTGCG CCGTGTGCGC

27401

27501

27601 CCGTGTGCTC TCACTGTGAT TTGCACTGTT CTTAAGCCCTG GATTCATCTA ACATCTTTCT TCCCTCTCTT GTCCTGATTA TAATAATATC AGAATTAATA
GGAACACAG AGTCACACTA AGCTTTTACA GATTTGGGAC CTATTTTACT TCTAGAACCA AGCGTATAGA CACGACTCAT ATTATTTATG TCTTTTAT
ATATACTGCG GCTCTCTATG CCATCTCTGA AACTGCGCG ATTTTACCGG CCAAGCGGAA CCTTTGCTCG TACTTTTAACT ATCTCTCC
TATATGAGCC CGAGGATAGC GGTAGGACAT TTGCGGTGCG AGAATGCGC GGTTCGCTTT GGAATGAGCC ATGAAATGAG TGAAGAGGTT
CTGTGATTTA CAACGCTTTC AAGCGAGAGC GACTGATCTT ACTGATGATC CTCTCGGAG CTTCTGAGCC TCAAGCTACT CATCTAGAAA AACACACCC TCTTTACCTT
GACACTAAAT GTTGTCAAG TTGGGTCTGC CTCACTCAGA TCTCTCTTTG GAGAGGCTCG AACTGATGAG GTAGTCTTTT TTGTGCTGCG AGGATAGAG
CGCGAAGCTT ACAGTGCCTT CAGCGCGCGC TTGACACAGC CTACCGCTG AGCTTTTTC GCGACAGAGC TCAATAAGCT TCTTTACCAT TCTTTACCAT
GCGCGCTTCA TGTCTACCGA GTGCGCGCGC GTGCGCGCG GATGCGGAG TGGCATTTG TCTGAAAGAG GCTCTCTCTG AGTTATGAG AGAATGCTC
AACAGAGCTT GAGCTTAGAA ACCCTTAGG GTATTAGCG GTATTAGCG AATGAGCGAG CTACTGTCTG GTTTATGAGC AATTCAGGCA ACTCTACGCG CTATTTCTAT
TTGTCTCTCA CTCGATCTT TTGGGAATCC CATATGCGCG TTTCGCGCTC GATGAGCCGC CAATTTCTTG TTATGCTGCT TCGATATGCGG GATGAGCTT

27701

27801

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28001

28101 TCAAGTTTCT CTGCAATGCG GUTTGCGGTT ATTCTCTGTC TTGTGATCTT CTTTATCTT ATACTTAAGC TTCTTGTGCT AAGGCTGCGC GCGTCTCT
AUTOCAGAGA GATCTTAGCC CCAACCGCCAA TAAGAGAGAG AACACTTAGA GAATATAGAA TATGATTTGCG AAGAGAGCGA TTCCGAGCGG CCGAGCAGAC
TGCACATTTG CATTTATTTT CAGCTTTTFA AAGCGTGCGG TCGCCACCCA ACATGATTAG GTACATAATC CTAGGTTTAC TCAGGCTTTC GTACGCTCAC
ACGTGTAAAC GTAAATACCA GTCGAANAAT TTGCGAGCGC ACGGTGGGCT TCTACTTAATC CATGTATTTG GATTCAGAAATG AGTGGAGAGC CAGTCCGCTT

28201

28301 GATACCCAGC AAAGGTGGA TTTTAAGGAG CCAAGCTGTA AGTTTACAT CCGAGCTGTA GTGAGCTGA GCTATGATCT GATTAATGCT ACCACAGT
CGATGCTGCG TTTCACACTT AAATTTCTTC GGTGCGGACAT TACATGTTAA GCTTCCGACTT GATTTACTCA CTGTGTGAGA ATATTTTACG TGTGTCTTT
ATGAAAGCT GCTTATGCG CACAAAGCA AAATTTGCGA GTATCTGTT TATGCTATTT GTGCGCAGG TGCACACTCA GAGTATATG CTGATATTTG AATGTCAAAA
TACTTTTTCG CCAATAGGCG GTGTGTTTTT TTATACGCTT CATACGACAA ATAGCATANA CCGTCCGCTC ACTGTGATGT CTGATATTTG AATGTCAAAA

28401

28501 CCAAGGTAAA AGTCATAAA CTTTATGTA TACTTTTCCA TTTTATGAAA TGTGCGACAT TACCATGTAC ATGAGCAAGC AGTATAGCTT GTGTGCGCA
GATCCCATTT TCAGTATTTT GAAATATCAT ATCAAAAGGT AAATATCTTT ACAGCTGTA ATGTATCATG TACTCTTTTG TCATATTTCA CACCGTGTGT
CAAAATGCG TCGAAGACAC TGTGCTTTTC TGTGCTACTG TGTGCTACTT TACAGTCTC GCTTTGTGCT GTACCGTACT GTATATTTAA TACANAGCA
GTTTTTAAGC ACCCTTTGCG ACCGTGAGAG AGAGCTGAG CCAAGGATTA ATGTACGAG ATGTACGAG CCAACCTAGA CATGATATTT ATGTTTCTT
GACGAGCTT TATTCAGAA AAGAAATATC CTTAATTTAC TAACTATGTA AGCTAATGTC AACTATGCT CCGTFACTCG GCTTTGCTAA ACNAAATTT
CTGCGCGAA ATAACTCTTT TCTTTTACG GAATTAATTC ATTCAGTTT TCCATATAG TCCGATTTGA CGAATATGAG GAGGAGCTT GTGTTTAT
AAAGGTTAGC ATTAATTTA GAATAGAT TTAAAGCCCG GGTCAATTTT TCTCTAATAC CATTCGCTCG AAGAAATGAG TCTATGTGCG ATATGCTCTA
TTTTCATGCT TAATATTAAT CTTATCTTAA ATTTGCGCGG CCAATTAAGG ACAGTTTATG GTTAGGAGAC TTGTTTACTG AGATACAGC TATACGAGCT

28601

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Figure 15R

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20901 GCGCTACGAC CTTGAACTCA GCTTCTCTTA ATGTAGCAT CTACTTTCTG CAGGACCTGG TGTTCAGAT TGTTCACAG CCACTACAG CCACTCCACT
 CCGCATCTTG GAATCTCAGT CCGTAAGATTC TACAGCTCTTA GACTGAAACC GATCTGTATC AGGCTCTCTA ACAAAGCTCA GGTTCATCTC GCTTCTCTTA
 29001 TACACAGAT GACCAACACA ACCAAGCTGG CCGCTCTCTAC CCGATCTTACA TTACACACA ATACACCCA AGTTCTCTCC TTCTCTCAAT ACTGCTATTA
 ATTCTCTCTA CTGCTCTCTG TGTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG
 29101 CTTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG TGTCTCTCTG
 GAACTCTCTG ACCCAACAGA GGTATCTCTG ATATCTCTTA TGTCTCTCTG CATCTCTCTG CATCTCTCTG CATCTCTCTG CATCTCTCTG CATCTCTCTG
 29201 TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 ATATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29301 CATCTCTCTG CCACTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29401 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29501 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29601 CCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 GCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29701 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29801 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 29901 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 30001 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 30101 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 30201 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 30301 GCGCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG
 CCGATCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG TATCTCTCTG

Figure 155

pHRAKd5qmg HPR682

30401 AATTTCTCT CCAGTTTAT CCCTCTAGCT CTGTATTTC AGCTCTCC TGGCTCCAA CTCTCCAC ATCTAAATG
 TTTAAGACA GGTCAATTA GTCTCTGTG AGTAAGGGA GATAGTGA GATACAGCTT ACCAGCTTT GAAAGAGTG TTAGATTTAC
 30501 GATATCTAGT TCTCTCTGT CCCTACCCAC TATCTCTAGT TGTGTTTGA TGAAGGTGTC ATAGATAGCT TCAACCCCT
 CTACAGTCA AGGAGGACA AGGACAGGTA GTATGTTTGT ATAGATGTC ACAGAGCTTC TCTGAGCA CTCTATGGA TGTATGGA A
 30601 GTATCCATAT GACAGGAAA CCGCTCTCC CAGTCTCTCT TTTCTACTC AGCTCTTGT ATCTCCCAAT GGTATCCAG AGATCTCCCT TGGGTACT :
 CATAGATATA CTGTCTCTT GGGCAGGAG TTAGACAGTA AAGAGATG GAGGAGACA TGGGGGTTA CCAAGATTC TCTCAGGGG ACCCCATGNG
 30701 TCTTTCCGC TATCCGAAC TCTATTACC TCTATTGCA TCTTTGCT CAAATGTC AGCTCTCT CTCTGAGCA GGGCGGCAC CTTACTCTC
 AGAAGGCGG ATAGCTTTG AGATCAATG AGTTTACCT AGTAAGCTA GTTTTACCG TGGCGGAGA GAGACCTGCT CCGGCCCTG GAATGAGI :
 30801 AATATTAAC CACTGTAGC CCACCTCTCA AAAAAACAA GTTAAATATA TATCTTACC CTTCAAGAT ACCCTAGAG CCGTACTCT
 TTTTACATT GTAGACTCG GTTGTGAGT TTTTGTGT CAGTTGTAT TGTACCTTT ATAGAGCTCA TGGATGCTC TGGATGCTC GGGATGACA
 30901 GGTTCGCCG GACCTCTAA TGGTCCCGG CAACACATC ACCTGCTC CACAGCCCG CTAAGCCGT CAGACTCCA AACTTAGCAT TCCACCCCA
 CCGACGCGG CGTGAGAT ACCAGCGCC GTTGCTGAG TGTAGCTTA GTTCCCGG GATTTGCC CAGTTGCCAC GTCTGAGT TGTATGCTA AGGTGGT :
 31001 GAGCCCTCA CAGTCTGAG AGAAGCTA GCGCTGAAA CACTACGCC CATCAAGCA GTACCTTAC TATCAGTCC TACCCCCCT
 CTTGGGAGT GTACAGTCT TCTTTGAT CCGAGCTT GTATGCGG GAGTGTGT TGGCTATCT CATGAGATG ATAGTACCG AGTGGGGA
 31101 TAACTACTC CACTGTAGC TTGGCATG AACCGTAA CTTGTAAAG GCGCATTTAT ACAGAAATG GAAACTAG GGGCTCTCT TGTATGTA
 ATTATATG GTAGCTTAC CCGCTTAA TGAATCTCT CCGTAAATG TGTGTTTCT GTTTGATCT TATTTATG TGGCGAGAA AGCTCATTA
 31201 AGAGAGCTA AGACTTGA CCGTAGCAC CCGTAGCAT GTATCTCT ATATCTCT CTTGCACT AGCTTAGT GAGCTTGG TTTTATCTA
 TCTCTGAT TGTGAACT GCGATGCT GCGATGCTA CACTGATAT TATTATGAG GAGCTTTGA TTTCAATGAC CTGGAACCC AATCTAT :
 31301 CAAGGAGTA TCGACTTAA TGTAGCAGA GAGACTTAA TTTATCTCA AAGAGAGCG CTATATCTG ATGTATGTA TCGTTTAT GCTCAACG
 GTTCCCTAT AGCTGATAT CACTGCTCT CCGTATCT CACTAGAT TTTCTCTG GATATGAG TACATCAAT AGGCAACTA CAGTTTAT
 31401 AACTAAATC AGACTAGGA CAGGCGCT TTTTATAA CTACGCCAC AACTGAGTA TTAAGTACA CAAAGGCTT TACTTGTTA GAGCTTCAA
 TGTATTAGA TCTGATCT GTCCCGGAG AATATATT GAGTGGGT TGTAGCTAT AATGATGTT GTTTCCGAA ATGACAAAT GTGAGATTT
 31501 CAATTCGAA AGCTTGA GCTCTTAG CACTCCAG GGTGATGT TTGAGCTAC AGCTTAGC ATTAATGAG GAGATGCT TGAATTTG :
 GTTAGGTT TCGAACTC AATGAGTC GTGAGCTTC CCGACTTCA AACTGAGT TGGTATG TGAATGCT CTCTACCGA ACTTAACCA
 31601 TCACCTAAT CAGCAACAC AATCCCTC AAGCAAAA TTTGCAATG TTTGCAATG CTTAGAAAT GTTCAACA AGCTATGAT TCTTAACCTA GGAAGCTC
 AGTGAATAC GTGTTTGT TTAGCGAG TTTGTCTTT ACCGTACC GATCTTAA CTAGTTGT TCGATAGCA AGGATTTGAT CTTGAGCTG
 31701 TTAGTTTGA CAGCAGAT GCAATTAG CCGTATGTC AATATGAT AAGCTAAGT TTTGAGCTC ACCAGTCCA TCTCTAAT CTAGACTTAA
 AATCAAACT GTGTTTGA CCGTATGTC ATCTTTGT TTTATCTA TTTGATTA ACCTGCTG AGCTAGAT AGAGATTTA CATCTGATTT
 31801 TCGAGGAAA GATCTTAA TCACTTGT CTTACAAA TGTGCTAG TTTGCTAG TACTTTCTA GTTTGCTG TTAGGCGAG TTTGCTGCA
 AGCTCTCT CTACGATG AGTGAACA GATTTGAT ACAGCTAG TTTATGAG TTTATGAGT CAAGCGCG AATTCCTC AAGCCAGGT
 31901 ATATCTGAA CATTCTAG TGTCTAGT ATTAAGAT TTTAGAAA TTAGTCTA CTAAACAT CTCTCTGGA CCGAGATAT TAACTTTA
 TATAGACT GTCAAGTTC AGTAGTAA TATATCTA AACTCTTT ACTTACAT CATTGTTA GGAAGAGCT GGTCTTATA ACCTTAAAT
 32001 GAAATGAAA TCTTACTA GCGCAACT ATCAAAAC TTTGATTT ATCTTACC TATCAGCTA TCCAAATCT CAGCTTAAA CTGCAAAAG
 CTTTACTCT AGATGACT CCGTCTGGA TATTTTCT AATCTTAA TACTGATG ATAGCTAT AGTTTAGA GTCCATTTT GAGCTTTTC

Figure 15T

pHINKA159ag MPR602

32101 TACATTTTC AGTCAGTTT ACTTAACCG AGCAGAACT AACCTTTTA CACTAACCT TACTAACCT GTACACAGG AACAGGGA CACAACTCA
ATCTTAACAG TCAGTTTTC TCAATTTTC TCTTTTTC TTTTACACT GTATTTTTC ATGTATTTTC CAAATTTTC TTTTCTCT GTCTTTTTC

32201 AGTCATAT CTATTCAT TCTATTCAG TCTATTCAG ACATTTAT TATTTTAT TATTTTAT TATTTTAT TATTTTAT TATTTTAT
TCATATAT GATACATTA AGTACCTTC AGTACCTTC TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32301 AATAAGAA CTATTCAT TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG
TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32401 GCTATTCAG ATCAGCTTC CTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32501 GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32601 ATAACTTC CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
TATTTTTC GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32701 AAGTTCAG CTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG
TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32801 CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

32901 TCTATTCAG CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
AGTAAATTA GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

33001 AAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

33101 CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

33201 AAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

33301 CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

33401 AAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

33501 GAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT CAGCAGCT
GCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG TCTATTCAG

Figure 15U

pHRK015gag MER6R2

35301 CATTTTAAAG AACTACAAAT TCCCAACACA TACAAATTTAC TCTCCCTTAA AACTACATTC ACCCGCCCTCC TTCCCGCCCG CCGCGCCACG TCCCAACATC
 GTAAATTTCT TTTCATATTA AGGTTTCTGT ATGTTCAATG ATCTGTAAT TTGCATCCAG TTGAGCGCCG MAGGTTCCCG GCGCGCCCTG AGTGTTTGAG

Pad
 ~~~~~  
 FcR1  
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35401 CACCCCTCA TTATCAATTT GCTTCAATC CAAATATAG TATATATTG ATATATTAA TTATGATTC GATCTCCGA CCGTAAGCTG GATGCTCTT
 GTGGGGAGT AATAGATTA CCGAATTTAG GTTTTATTC ATATAATTA TACTACATTT ATTTCTTAAG CTTAGAGCTT GCGCTCCGAC CTACCTGAAAG

35501 CCCATTTAA TTCTTCTCG TTCCCGCGCG ATCGGATTC GCGGTTTGG TCGATAGCGA GCGATACCGA TATATACCGA CAGTACGGGA CAGCTTCAAG

35601 GGTAAATACT AGAAGAGCG AGGCGCGCG TACGCTTCT GCGGTTTTC GATAGCTCC GCGGCGGACT GCTGTTAGTG TTTTTAGCT CAAATTTAGT
 GCTACGAAA GCGCGGAAAC GTTAAAAAGT CCGCTTCTT GCGGTTTTC GATAGCTCC GCGGCGGACT GCTGTTAGTG TTTTTAGCT CAAATTTAGT
 CCGTCTTTT CCGTCTTCTT GATTTTCTT GCGGTTTTC GCGGTTTTC GATAGCTCC GCGGCGGACT GCTGTTAGTG TTTTTAGCT CAAATTTAGT

35701 GAGTTGCGGA AACCTGACAG GACTATTAAG ATACCAAGCG TTTCGCGCTT GAGCTCTCTT GAGCTCTCTT GAGCTCTCTT GAGCTCTCTT GAGCTCTCTT
 CTCACCGCTT TTGCGCTTTC GCGGAGCTTT GATGTTTTC TATGTTCCG ATAGTGGAG TTTCGCGCTT GAGCTCTCTT GAGCTCTCTT GAGCTCTCTT

35801 GACAGCGGGA AGGAGCGGAG CCGTTCCGAC CCGGAAAGT GCGGTTTTC GATAGCTCC GCGGTTTTC GATAGCTCC GCGGTTTTC GATAGCTCC
 TCCAGGAAC CCGCTTTCAG CCGGAGCTT CCGGTTTTC GCGGTTTTC GATAGCTCC GCGGTTTTC GATAGCTCC GCGGTTTTC GATAGCTCC

35901 ACCTGCTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC GCGGTTTTC
 CACTGTTAAC AGATTAAGTA GAGCGAGGTA TGTAGCGCTT GGTACAGCTT GGTACAGCTT GGTACAGCTT GGTACAGCTT GGTACAGCTT

36001 GTAGGCAATG TCTTAATCTT CTGCTCCAT ACATCGCGA CCAATGCTCA AGAATTTTC CACCGGCTT TCTGTTTTC TCTGTTTTC TCTGTTTTC
 ATCTGCGCTC TCTGTTTTC AGTTTCTTC GGAAGAGAG TTCTGCTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC

36101 TATAGCGGAG AGCACTTCTG TCAATGAG CTTTCTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC
 AGCAGCGAGT TACGCGGAG AAAAAAGAT CTCAGAGAG TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC TCTGTTTTC

36201 TCTGCTCTTA ATGCGCTCT TTTTCTCTA GAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT
 TTGCTGATG AGATTATCA AAGGATCTT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT CAGTTCTCT

36301 AACCAATAC TCTAATAGTT TTTCTAGAA GTGAGCTAG GTGAGCTAG GTGAGCTAG GTGAGCTAG GTGAGCTAG GTGAGCTAG GTGAGCTAG
 TCAATGAGC ACCTATCTA CCAATCTCT TATTTCTTC TATTTCTTC TATTTCTTC TATTTCTTC TATTTCTTC TATTTCTTC TATTTCTTC

36401 AGTCACCTCC TGGATAGAT CCGTAGACAG ATAAAGGAG TGGATATCA TGGATATCA TGGATATCA TGGATATCA TGGATATCA TGGATATCA
 TGGCGGAGT GCTGCAATGA TACCGCGAGA CCGAGCTCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT

36501 ACCCGGCTCA CCACTTACT ATGCGCTCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT
 CCGGCGGCT TATCGGCTC CAGCGAGCT ATTAATGTT TGGCGGAGT TGGCGGAGT TGGCGGAGT TGGCGGAGT TGGCGGAGT TGGCGGAGT

36601 GATCGGCTTA ATAGCGGAG CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT
 CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT

36701 CTAAGGCTAT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT
 GATGCTCTTA CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT

Pad
 ~~~~~

36801 AAGAGCGGTT AGCTCTCTTC GTCTCTCTAT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT  
 TTTTCCGCAA TCCAGGAGCG CAGGAGCTTA CCAATCTCT TCAATCTCT TCAATCTCT TCAATCTCT TCAATCTCT TCAATCTCT TCAATCTCT

36901 GTGATGCTAT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT  
 CAGTACGCTTA CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT CCGGCGGCT

figure 15W



pmRNA15qng MER682

37001 CACACCGGA TATACCGCG CCACATAGCA GAACCTTAAA AGTATCATC ATGTGAAAC GTCTTCGGG GCGAAUACTC TCAGCATCT TACTTCTCTT  
 GTTGTGCGCT ATTATCGCG CCCTATATCT GTTGAAATTT TTACCACTAG TACCTTTTG CAGCAGCCG CCGTTTTCAG ACTTCCTAGA ATGCGACAA  
 37101 GAGNTCCAGT TCGATTTAAC CCACTCTGCG ACTCAATCTA TCTTAACTAT TTACCTTTT CACTTACCTT TCGAGCTGAG CAAAAACAGG AACGCAAAAT  
 CTCCTAGCTCA AGCTACATCG GTTCAGCAGC TCGCTTGACT AGAATCTCTA GAATATGAAA GTTCTCTCAA AGACCCACTC GTTTTTCGCC TTCCCTTTTA  
 37201 GCGCCAAAAA AGGATATAG GCGGACAGCG AATGCTGAAA TACTTATCT TTCTCTTTT CAAATATTT GAGCATTTA TCAGATTTAT TCTCTCATCA  
 CCGCTTTTTT TCCCTATTC CCGCTCTGCC TTACAACTT ATCAGTATCA GAATATGAAA GTTATATATA CTTCGTAAAT AGTCCCAATA ACAGATATCT  
 37301 GCGGATACAT ATTGATGT ATTAGAAA ATAAACAAAT AGGATTTTG CTACATTTT CCGCAAAAT GCCACCTGAC GTCTAGAAA CCAATTATTA  
 CCGCTATGTA TAACTTACA TAACTTTTT TATTGTTTA TCCCCAGCG CCGGTAAAG GCGCTTTCA CCGTGGACTG CAGATCTCTT GGTATATATA

37401 CATGACATTA ACCTATAAA ATAGCGTAT CACTAGCGCG TTCTGCTTC AATATGGA TTGCAATCT TAAAT (SEQ ID NO: 27)  
 GTTCTGTAAT TGGATATTTT TATCCGATA GTCTCCCGG AAGCAGAG TTCTTACCT AGCCTAGAA ATTA (SEQ ID NO: 28)

8amHj  
 EcIII

Figure 15X

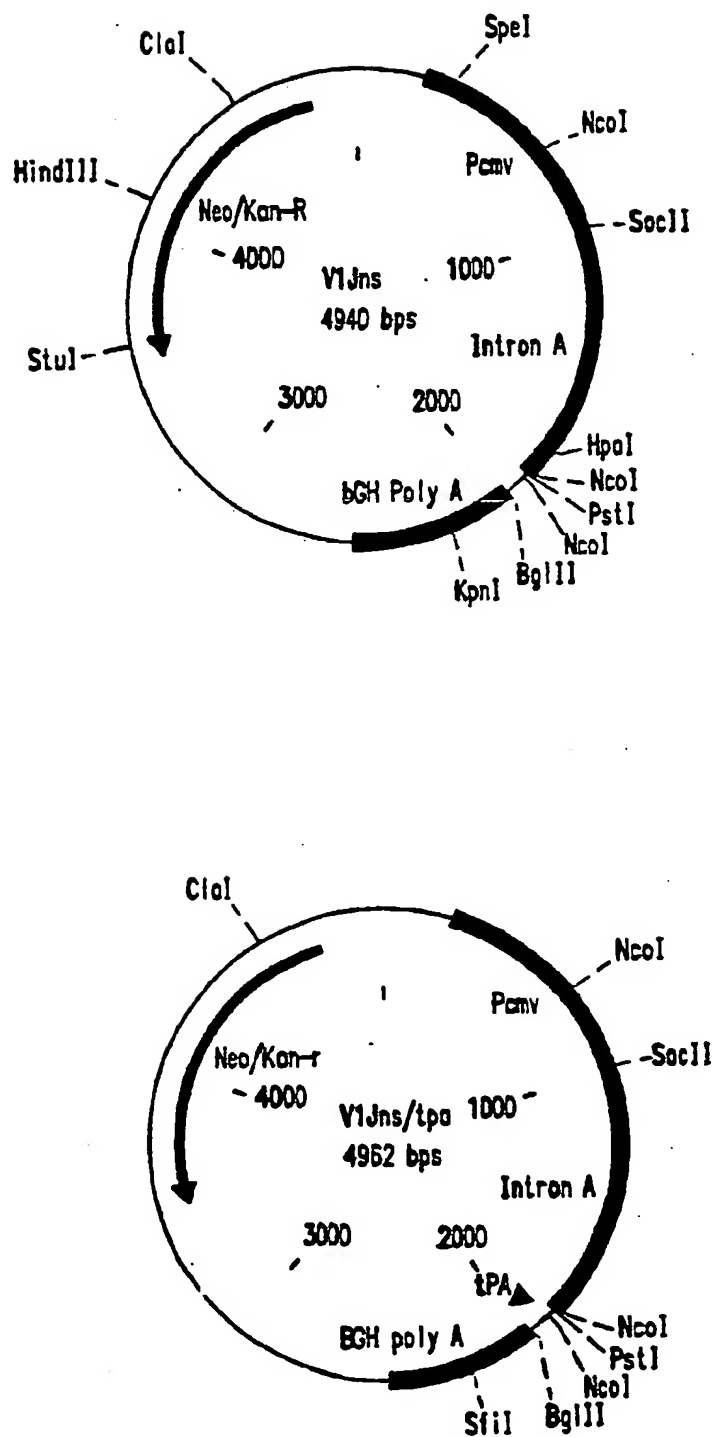


FIGURE 16

AGATCTACCATGGCCCCATCTCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGTGAA  
 Bg/II MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLy  
 1 10 20  
 GCAGTGGCCCCGTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGCAAAATCTCCA  
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL  
 30 40 50  
 AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG  
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal  
 60 70  
 GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA  
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLy  
 80 90 100  
 GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG  
 sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA  
 110 120 130  
 CCTTCACCATCCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCACTACAATGTGCTGCCCCAGGGCTGSAAGGGC  
 loPheThrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly  
 140 150  
 TCCCTGCCATCTTCCAGTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA  
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGl  
 160 170 180  
 GTACATGGCTGCCCTGTATGTGGCTCTGACCTGGAGATTGGGACGACAGGACCAAGATTGAGGAGCTGAGGCAGCADC  
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL  
 190 200 210  
 TGCTGAGGTGGGGCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGGCTATGAGCTGCAC  
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis  
 220 230  
 CCGACAAGTGGACTGTGCAGCCATTGTGCTGCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG  
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGl  
 240 250 260  
 CAAGCTGAAGTGGGCTCCCAAATCTACCCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC  
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaI  
 270 280 290

FIGURE 17A

TGA CTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT  
 E d Thr Glu Val I l e Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu I l e Leu Lys Glu Pro Val His  
 300 310

GGGGTG TACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCACTGGACCTACCAATCTA  
 Gly Val Tyr Tyr Asp Pro Ser Lys Asn Leu I l e Ala Glu I l e Glu Lys Glu Gly Glu Gly Glu Trp Thr Tyr Glu I l e Ty  
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCACACCAATGATGTGAAGCAGCTGA  
 r Glu Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Glu Leu T  
 350 360 370

CTCAGGCTGTGCAGAGATCACCCTGAGTCCATTGTGATCTGGGGCAAGACCCCAAGTTCAAGCTGCCCATCCAGAAG  
 hr Glu Ala Val Glu Lys I l e Thr Thr Glu Ser I l e Val I l e Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro I l e Glu Lys  
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT  
 Glu Thr Trp Glu Thr Trp Thr Glu Tyr Trp Glu Ala Thr Trp I l e Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Le  
 400 410 420

GG TGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATGTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG  
 u Val Lys Leu Trp Tyr Glu Leu Glu Lys Glu Pro I l e Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg G  
 430 440 450

AGACCAAGCTGGCAAGGCTGGCTATGTGACCAACAGGGCCAGGCAGAGAGTGGTGACCTGACTGACACCACCAACCAG  
 I l e Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Glu Lys Val Val Thr Leu Thr Asp Thr Thr Asn Glu  
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC  
 Lys Thr Ala Leu Glu Ala I l e Tyr Leu Ala Leu Glu Asp Ser Gly Leu Glu Val Asn I l e Val Thr Ala Ser Glu Tyr Al  
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG  
 a Leu Gly I l e I l e Glu Ala Glu Pro Asp Glu Ser Glu Leu Val Asn Glu I l e I l e Glu Glu Leu I l e Lys Lys G  
 510 520 530

AGAAGGTGTACCTGGCCTGGTGCCTGCCACAAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC  
 I l e Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly I l e Gly Gly Asn Glu Glu Val Asp Lys Leu Val Ser Ala Gly  
 540 550

ATCAGGAAGGTGCTGTTCTGGATGGCATTGACAAGGCCAGGATGAGCATGAGAAGTACCACTCCAAGTGGAGGGCTAT  
 I l e Arg Lys Val Leu Phe Leu Asp Gly I l e Asp Lys Ala Glu Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Me  
 560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCTCTGGTGGCTAAGGAGATTGTGGCTCCCTGTGACAAGTGCCAGCTGAAGGGGAGG  
 tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA  
 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG  
 lAlaMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal  
 620 630

GCTGTGCATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCCTGCT  
 AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe  
 640 650 660

GAAGCTGGCTGGCAGGTGGCTGTGAAGACCATCCACACTGCCAATGGCTCCAACCTCACTGGGGCCACAGTGAGGGCTG  
 uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA  
 670 680 690

CCTGCTGGTGGCTGGCATCAAGCAGGAGTTGGCATCCCTACAACCCCACTCCACGGGGTGGTGGCTCCATGAAC  
 lAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn  
 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT  
 LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle  
 720 730 740

CCACAACCTCAAGAGGAAGGGGGCATCGGGGCTACTCCGCTGGGAGAGGATTGTGGACATCATTGCCACAGACATCC  
 eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG  
 750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGACTACAGGACTCCAGGAACCCCTGTGG  
 lnThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp  
 780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGAGGGGCTGTGGTGATCCAGGACAACCTCTGACATCAAGGTGGTGGCCAG  
 LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr  
 800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT  
 gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx  
 830 840 850

AAAGCCCCGGCAGATCT (SEQ ID NO: 3)  
 Xx BgII (SEQ ID NO: 4)

FIGURE 17C

**FIGURE 18**

|     |                                                           |      |
|-----|-----------------------------------------------------------|------|
| WT  | - ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT | -42  |
|     |                                                           |      |
| OPT | - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC | -14  |
|     | M G G K W S K R S V P G W S                               |      |
| WT  | - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT | -84  |
|     |                                                           |      |
| OPT | - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC | -28  |
|     | T V R E R H R R A E P A A D                               |      |
| WT  | - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA | -126 |
|     |                                                           |      |
| OPT | - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC | -42  |
|     | R V R R T E P A A V G V G A                               |      |
| WT  | - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC | -168 |
|     |                                                           |      |
| OPT | - GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC | -56  |
|     | V S R D L E K H G A I T S S                               |      |
| WT  | - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA | -210 |
|     |                                                           |      |
| OPT | - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC | -70  |
|     | N T A A T N A D C A W L E A                               |      |
| WT  | - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA | -252 |
|     |                                                           |      |
| OPT | - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG | -84  |
|     | Q E D E E V G F P V R P Q V                               |      |
| WT  | - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC | -294 |
|     |                                                           |      |
| OPT | - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC | -98  |
|     | P L R P M T Y K G A V D L S                               |      |
| WT  | - CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC | -336 |
|     |                                                           |      |
| OPT | - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC | -112 |
|     | H F L K E K G G L E G L I H                               |      |
| WT  | - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC | -378 |
|     |                                                           |      |
| OPT | - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC | -126 |
|     | S Q K R Q D I L D L W V Y H                               |      |
| WT  | - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG | -420 |
|     |                                                           |      |
| OPT | - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC | -140 |
|     | T Q G Y F P D W Q N Y T P G                               |      |

FIGURE 19A

|     |                                                              |      |
|-----|--------------------------------------------------------------|------|
| WT  | - CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG    | -462 |
|     |                                                              |      |
| OPT | - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG    |      |
|     | P G I R F P L T F G W C F K                                  | -154 |
| WT  | - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA    | -504 |
|     |                                                              |      |
| OPT | - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG    |      |
|     | L V P V E P E K V E E A N E                                  | -168 |
| WT  | - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG    | -546 |
|     |                                                              |      |
| OPT | - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC    |      |
|     | G E N N C L L H P M S Q H G                                  | -182 |
| WT  | - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC    | -588 |
|     |                                                              |      |
| OPT | - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC    |      |
|     | I E D P E K E V L E W R F D                                  | -196 |
| WT  | - AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG    | -630 |
|     |                                                              |      |
| OPT | - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC    |      |
|     | S K L A F H H V A R E L H P                                  | -210 |
| WT  | - GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)                 | -651 |
|     |                                                              |      |
| OPT | - GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9) |      |
|     | E Y Y K D C (SEQ ID NO:10)                                   | -216 |

FIGURE 19B



VIJns/nef *PstI* *BglII*  
 CATGGGTCCTTTTCTGAGTCACCGTCTCTTGAAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC  
 M G G K W S K R S V P  
 . . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA *SrfI* *BglII*  
 AGCCGGGCGCAGATCTGCTGTGCTCTCTAGTTGCCAGC (SEQ ID NO: 38)  
 H P E Y Y K D C \* (contained within SEQ ID NO: 10)

VIJns/nef(G2A.LLAA)  
*PstI* *BglII*  
 CATGGGTCCTTTTCTGAGTCACCGTCTCTTGAAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC  
 M A G K W S K R S V P  
 . . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA *SrfI* *BglII*  
 AGCCGGGCGCAGATCTGCTGTGCTCTCTAGTTGCCAGC (SEQ ID NO: 39)  
 H P E Y Y K D C \* (contained within SEQ ID NO: 14)

VIJns/tpanef & VIJns/tpanef(LLAA)  
*PstI*  
 CATGGGTCCTTTTCTGAGTCACCGTCTCTTATATCTAGATCACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG  
 M D A M K R G L C C V  
 CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG *BglII*  
 ATC TCC TCC AAG AGG TCC GTG CCC  
 L L L C G A V F V S S E I S S K R S V P  
 . . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA *SrfI* *BglII*  
 AGCCGGGCGCAGATCTGCTGTGCTCTCTAGTTGCCAGC (SEQ ID NO: 40)  
 H P E Y Y K D C \* (contained within SEQ ID NO: 16)

FIGURE 20

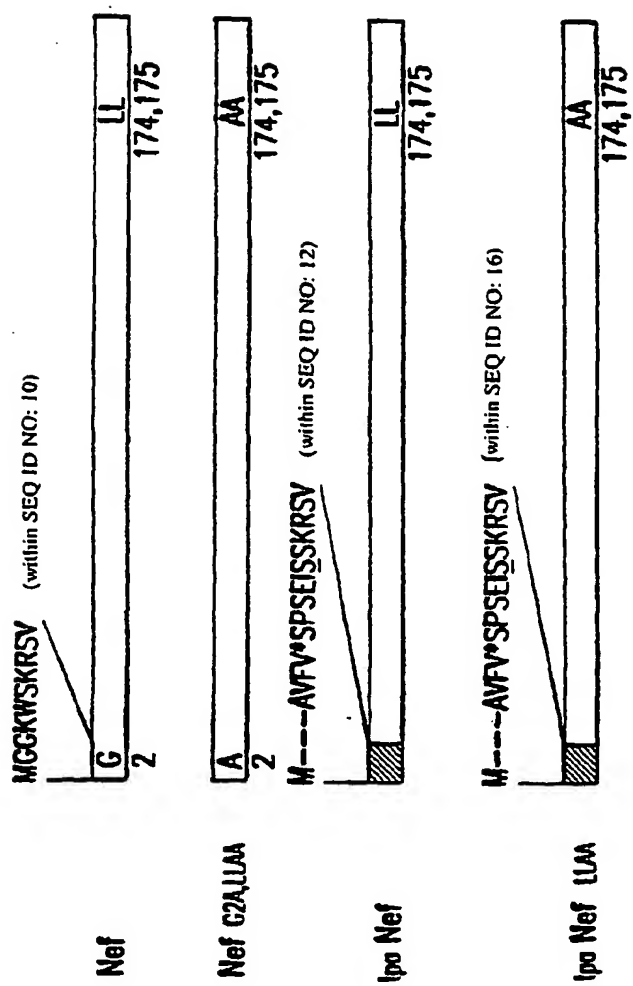


FIGURE 21

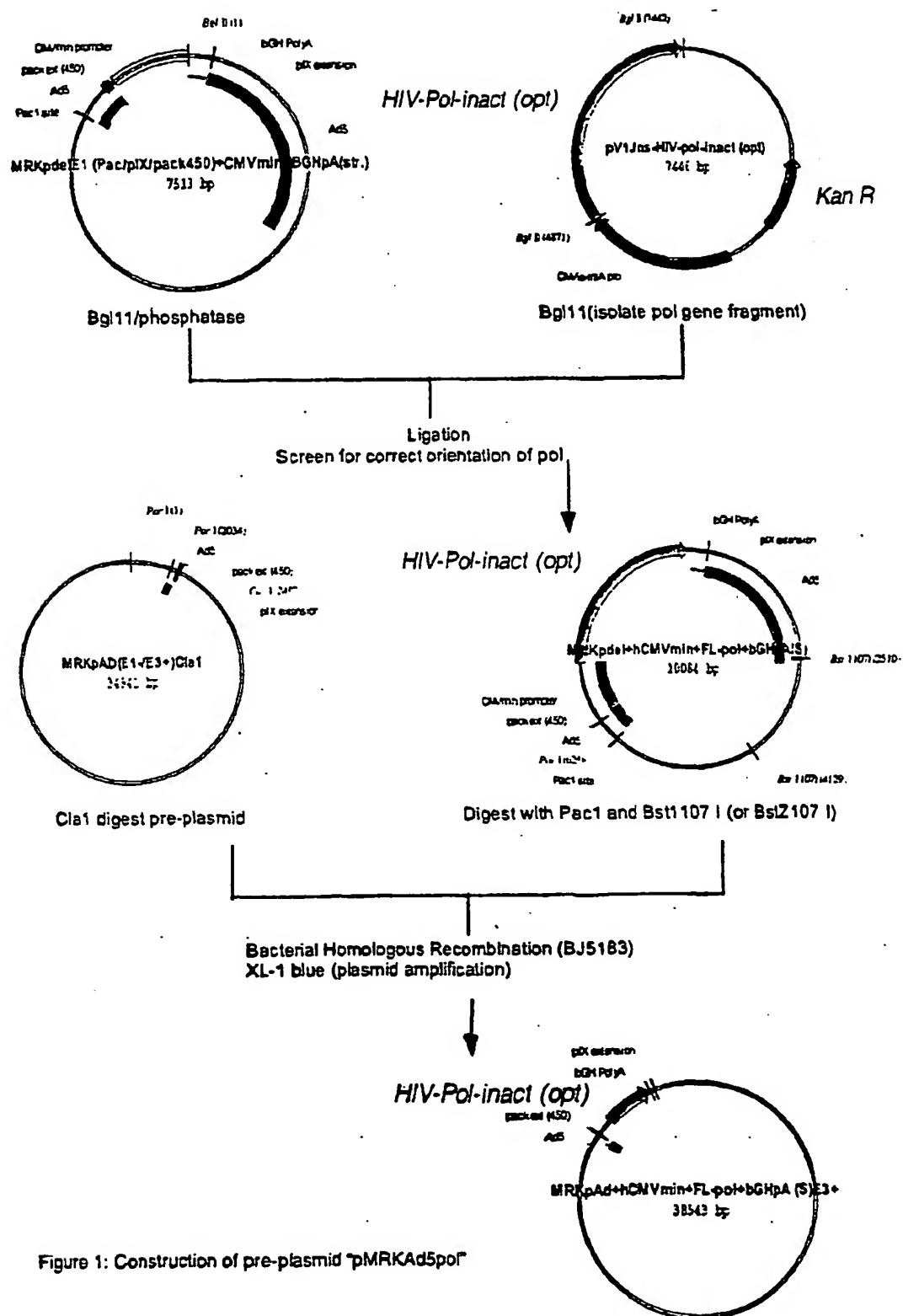


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22

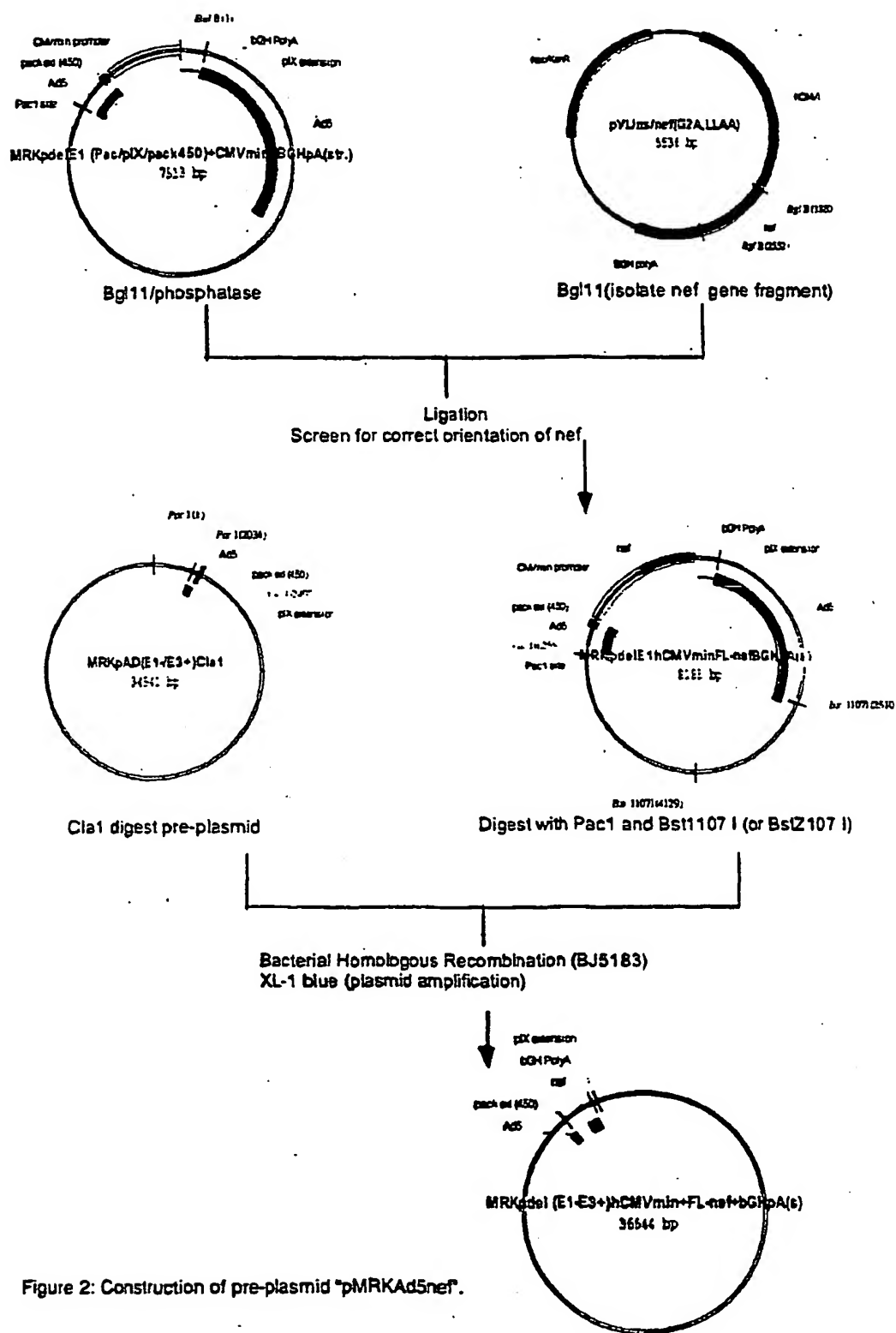
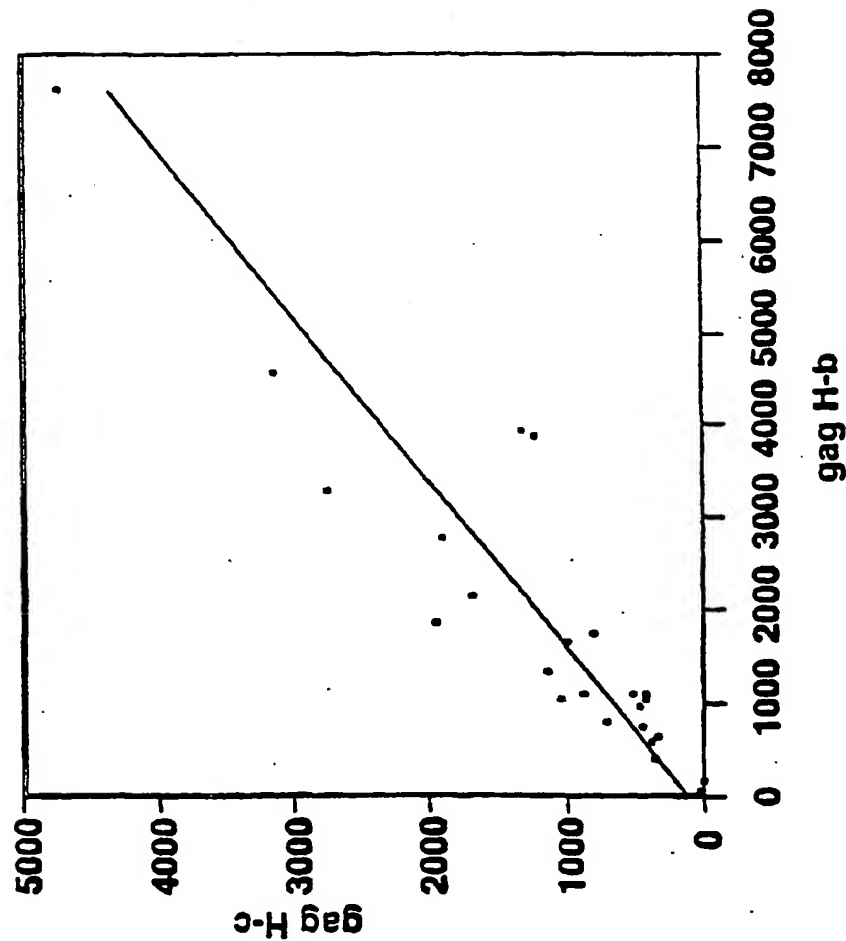


Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

# Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



|                                     |          |
|-------------------------------------|----------|
| Linear Fit                          |          |
| gag H-c = 111.603 + 0.55866 gag H-b |          |
| Summary of Fit                      |          |
| RSquare                             | 0.816775 |
| RSquare Adj                         | 0.80914  |
| Root Mean Square Error              | 474.9639 |
| Mean of Response                    | 1158.115 |
| Observations (or Sum Wgts)          | 26       |

# Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects

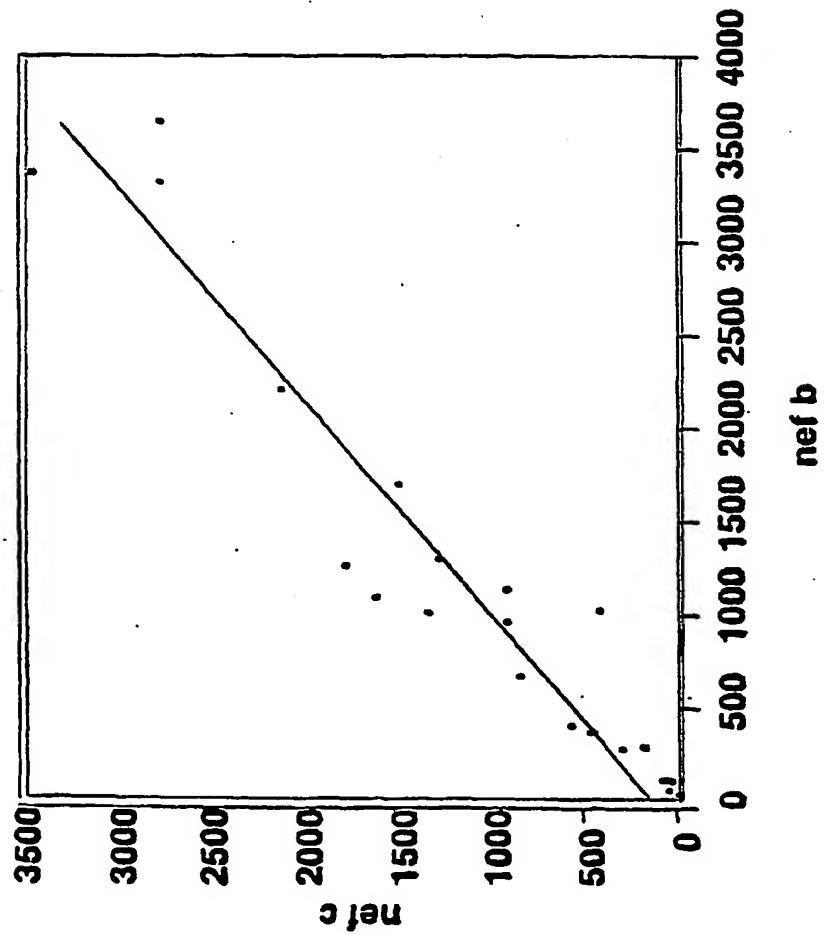


FIGURE 25

**MRKAd5pol MER1062**  
**(MRKAd5 Pre-Adenoviral Vector Containing the LA opt pol Coding Region)**

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGCGCAAG GCCCAGTTTC AACCGCAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCGGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCCGC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAATGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGCCCC GCCTGGCATT ATGCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAAT

851 CATGACCTTA TGGGACTTTC CTACTTGCCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCACT

```

*Figure 26A*

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTTGAGGCG GGGTAAGTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGGCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCAT TGAGACTGTG CCTGTGAAGC  
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG  
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG  
CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG  
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG  
GGTAGTTCTT CTTCTGAGG TGGTTCACCT CTTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC  
CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG  
GGTGGGGCGA CCGGACTTCT TCTTCTTCAG AACTGACAC GACCGACACC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT  
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTCATGTGA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA  
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT  
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT  
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 26B



1901 GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG  
 CCCC GGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC  
 1951 TGGATGGGCT ATGAGCTGCA CCCC GACAAG TGGACTGTGC AGCCCATTGT  
 ACCTACCCGA TACTCGACGT GGGGCTGTTC ACCTGACACG TCGGGTAACA  
 2001 GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG  
 CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC  
 2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC  
 2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT  
 GACACGTTTCG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA  
 2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG  
 CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC  
 2201 AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG  
 TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCTCTGA CTAACGACTC  
 2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC  
 TAGGTCTTCG TCCCGGTCCC GGTCACCTGG ATGGTTTAGA TGGTCTTCGG  
 2301 CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA  
 GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCC GGGTGT  
 2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
 GGTACTACA CTTGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC  
 2401 TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA  
 AGGTAACACT AGACCCCGTT CTGGGGGGTTC AAGTTCGACG GGTAGGTCTT  
 2451 GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC  
 CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG  
 2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG  
 GACTCACCCCT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC  
 2551 CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC  
 GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGAAGATAC ACCGACCCCG  
 2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG  
 ACGGTTGTCC CTCTGGTTTCG ACCCGTTCCG ACCGATACAC TGGTTGTCCC  
 2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
 CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG  
 2701 CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT  
 GAGGTCCGGT AGATGGACCG GGAGGTCTTG AGACCGGACC TCCACTTGTA  
 2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC  
 AACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

Figure 26C

2851 GAGAAGSTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA  
 CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCGTT  
 2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC  
 ACTCGTCCAC CTGTTCCGACC ACAGACGACC GTAGTCCTTC CACGACAAGG  
 2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
 ACCTACCGTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG  
 3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCTGTGG TGGCTAAGGA  
 ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTTCCT  
 3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG  
 CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC  
 3101 GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG  
 CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC  
 3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA  
 CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAATC  
 3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC  
 CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG  
 3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
 ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG  
 3301 TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT  
 AGGTGGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA  
 3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG  
 GTTCGTCTTC AAACCGTAGG GGATGTTGGG GGTCAGGGTC CCCCACCACC  
 3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG  
 GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCTGGTC  
 3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCT  
 CGACTCGTGG ACTTCTGTCTG ACACGTCTAC CGACACAAGT AGGTGTTGAA  
 3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG  
 GTTCTCCTTC CCCCCGTAGC CCCCAGTAG GCGACCCCTC TCCTAACACC  
 3551 ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
 TGTAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG  
 3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG  
 TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCCT TGGGGGACAC  
 3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC  
 CTTCCCGGGA CGGTTCGACG ACACCTTCCC CCTCCCCCGA CACCACTAGG  
 3701 AGGACAACCTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC  
 TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

*Figure 26 D*

3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC  
CCTACTCCTG ATTTCTGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG

3851 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC  
GTAGACAACA AACGGGGAGG GGGCACGGAA GGAAGTGGGA CCTTCCACGG

3901 ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT  
TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951 GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG  
CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTC

4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT  
CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051 ATGGCCGATC GGCGCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG  
TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCAC

4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC  
CCTTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA  
TCGTCCGGCG CGCGGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT

4201 GTCATATTTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT  
CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCC CAAACTCTAC  
CACTACCCGA GGTCTGTAAC ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT  
ATGGAAGTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA

4351 CCGCCGCCCG TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC  
GGCGCGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC  
AAACGAAAGG ACTCGGGCGA ACGTTTGTC ACGTCGAAGG CAAGTAGGCG

4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC  
GGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501 GGGAACTTAA TGTCTTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT  
CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTCTGTC

4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA  
AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT

4601 AAAACCAGAC TCTGTTTGGG TTTGGATCAA GCAAGTGTCT TGCTGTCTTT  
TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTGCG  
TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCTG CAGAGCCAGC

Figure 26E

4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT  
 CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA  
 4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG  
 CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC  
 4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC  
 CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG  
 4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTGA AGCTGGGATG  
 GTCCCCGTCC GGAACCAACA TTCACAAATG TTTGCGCAAT TCGACCCTAC  
 4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG  
 CCACGTATGC ACCCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC  
 5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC  
 CGATACAAGG GTCGGTATAG GGAGGCCCCCT AAGTACAACA CGTCTTGGTG  
 5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCATGT AGCTTAGAAG  
 GTCGTGTAC ATAGGCCACG TGAACCCCTT AAACAGTACA TCGAATCTTC  
 5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC  
 CTTTACGCAC CTTCTTGAAC CTCTGCGGGA AACTGGAGG TTCTAAAAGG  
 5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC  
 TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCGCC GCCGGACCCG  
 5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT  
 CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA  
 5251 CGTCATAGGC CATTTTTTACA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT  
 GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCCACGG TCTGACGCCA  
 5301 ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT  
 TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA  
 5351 TTCCACGCT TTGAGTTCAG ATGGGGGAT CATGTCTACC TGCGGGGCGA  
 AAGGGTGCGA AACTCAAGTC TACCCCTTA GTACAGATGG ACGCCCCGCT  
 5401 TGAAGAAAAC GGTTCGCGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG  
 ACTTCTTTTG CCAAAGGCC CATCCCTCT AGTCGACCTT TCTTTCGTCC  
 5451 TTCTGAGCA GCTGCGACTT ACCGCGCCG GTGGGCCCCG AAATCACACC  
 AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG  
 5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCGCTG CCGTCATCCC  
 ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG  
 5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC  
 ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG  
 5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTCTTGT  
 GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

Figure 26 F

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC  
 AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG  
 5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG  
 ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC  
 5801 CGGCTTTTCG TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT  
 GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA  
 5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG  
 GTACAGAAAG GTGCCCCGCT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC  
 5901 TGAAGGGGTG CGCTCCGGGC TGCGCGCTGG CCAGGGTGCG CTTGAGGCTG  
 ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC  
 5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG  
 CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC  
 6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT  
 CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGGGA  
 6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA  
 ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT  
 6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA  
 GAAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCTCAT  
 6151 GGCATCCGCG CCGCAGGCC CCGCAGACGGT CTCGCATTCC ACGAGCCAGG  
 CCGTAGGCGC GCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC  
 6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTCCTCCC ATGCTTTTGG  
 ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAC  
 6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC  
 TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG  
 6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCCTCGA  
 CTTTTCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT  
 6351 GCGGTGTTCC GCGGTCTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA  
 CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT  
 6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG  
 TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTACCC TCCCCATCGC  
 6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT  
 CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACACT TCTGTGTACA  
 6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG  
 GCGGGAGAAG CCGTAGTTCC TTCCAATAAC CAAACATCCA CATCCGGTGC  
 6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGTGG GGGCGCGTTC  
 ACTGGCCCCA AAGGACTTCC CCCCGATATT TTCCCCACC CCCGCGCAAG

Figure 266

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAAGT  
 TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTG TAACAGTCAA  
 6701 TCCAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT  
 AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA  
 6751 GAGGGTGGCC GCATCCATCT GGTGAGAAAA GACAATCTTT TTGTTGTCAA  
 CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAAACAGTT  
 6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG  
 CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC  
 6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT  
 CTCGCGTCCC AAACCAAAAA CAGCGCTAGC GCGCGGAGGA ACCGGCGCTA  
 6901 GTTTAGCTGC ACGTATTCGC GCGCAACGCA CCGCCATTGCG GGAAAGACGG  
 CAAATCGACG TGCATAAGCG CGCGTTGCGT GCGGTAAGC CCTTCTGCGC  
 6951 TGGTGCCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG  
 ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTGGCGC CAACACGTCC  
 7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT  
 CACTGTTCCA GTTGCACCA CCGATGGAGA GCGCATCCG CGAGCAACCA  
 7051 CCAGCAGAGG CGGCCGCCCT TCGCGAGCA GAATGGCGGT AGGGGGTCTA  
 GGTCGTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT  
 7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC  
 CGACGCAGAG CAGGCCCCCC AGACGCAGGT GCCATTTCTG GGGCCCGTCC  
 7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCACT CCTTGCAAGT CTAGCGCCTG  
 TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTCA GATCGCGGAC  
 7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC  
 GACGGTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCTG  
 7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTGG  
 GGGTACCGTA CCCCACCCAC TCGCGCTCC GCATGTACGG CGTTTACAGC  
 7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT  
 ATTTGCATCT CCCCAGAGAG CTCATAAGGT TCTATACATC CCATCGTAGA  
 7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTGCG TGCGAGGGAG  
 AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGTCCCTC  
 7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG  
 GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTC  
 7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG  
 TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC  
 7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG  
 CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

7601 TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG  
 AGATCCC CGC TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC  
 7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT  
 AGGGAAAAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA  
 7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT  
 AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA  
 7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC  
 TCGTACATCT TGACCAACTG CCGGACCATC CCGCTCGTAG GGAAAAGATG  
 7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG  
 CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC  
 7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG  
 GTTCCACAG GGA CTGGTAC TGAAACTCCA TGACCATAAA CTTCAGTCAC  
 7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTGGGA  
 AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT  
 7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG  
 TGCGCCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC  
 8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA  
 GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT  
 8051 CGGTGTGTTAA TTACCTGGGC GGCAGACAG ATCTCGTCAA AGCCGTTGAT  
 GCCAACAATT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA  
 8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG  
 CAACACCGGG TGTTACATTT CAAGGTTCTT CGCGCCCTAC GGGAACTACC  
 8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC  
 TTCCGTTAAA AAATTCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG  
 8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA  
 GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCAACC TTCGCTGCTT  
 8251 TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG  
 ACTCGAGGTG TCCAGTGCCC GGTAAATCGTA AACGTCCACC AGCGCTTTCC  
 8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG  
 AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC  
 8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTCC CGGCTAGGTC  
 CATTCGCCCC GAACAAGGGT CGCCAGGGTA GGTCCAAGC GCCGATCCAG  
 8401 TCGCGCGGCA GTCCTAGAG GCTCATCTCC GCCGAAC TTC ATGACCAGCA  
 AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT  
 8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCC CCATCCAAGT ATAGGTCTCT  
 ACTTCCCGTG CTCGACGAAG GGTTCGGG GGTAGGTTCA TATCCAGAGA

Figure 26I

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT  
CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA

8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTAA  
CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT

8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG  
TTTGACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCCT  
CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCCTTA AACTCGGGGA

8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTCCGGCTGC TTGTCTTTGA  
GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT

8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG  
GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGGCG

8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTCGGAGC TTGATGACAA  
GCTCGGGTTT CAGGTCTACA GCGCGCGGCC GCCAGCCTCG AACTACTGTT

8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG  
GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG  
AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC

9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT  
CCGATCTAGG TCCACTATGG ATTAAAGGTC CCGACCAAC CACCGCCGCA

9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC  
GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG

9101 GCGGGGCGST GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG  
CCGCCCCGCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG  
ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GCGGCCCCTC

9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG  
TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCCTCG ACCACGACGC

9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GGCGGTTGAT CTCCTGAATC  
GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG

9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCC GTGAGCTTGA ACCTGAAAGA  
ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT

9351 GAGTTCGACA GAATCAATTT CCGTGTCGTT GACGGCGGCC TGGCGCAAAA  
CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCCGCGG ACCGCGTTTT

9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC  
AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J



9501 GCGGCGGAGG TCGTTGGAAA TCGGGGCCAT GAGCTGCGAG AAGGCGTTGA  
 CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT  
 9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG  
 CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC  
 9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA  
 GCCCGCGCGT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCCGTT  
 9651 GACGGCGTAG TTTGCGAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG  
 CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC  
 9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTCTG  
 ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC  
 9751 TTGATATCCC CCAAGGCCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC  
 AACTATAGGG GGTTCGCGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG  
 9801 GGCGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT  
 CCGCTTCAAC TTTTGTACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA  
 9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG  
 GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC  
 9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC  
 CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG  
 9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC  
 GGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCGGCTG  
 10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG  
 CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC  
 10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG  
 GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC  
 10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTGGGC GGGGGGCTGC  
 AACCTTCTGC GGCGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCGACG  
 10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA  
 GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT  
 10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA  
 CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCTAGCCT  
 10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAGGCTGA  
 TTTGGAGAGC TCTTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT  
 10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTGTT TCTGGCGGAG  
 CGTGGCACCG CCCGCCGTCG CCCGCCGCA GCCCAACAA AGACCGCTC  
 10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GGCGGATGGT  
 CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTAGTAG  
 GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC  
 10501 TCTTGCATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTTGTCC  
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG  
 10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT  
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAAA CCGGCATCCA  
 10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCCT CATCGGCTGA  
 CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT  
 10651 AGCAGGGCTA GGTGCGCGAC AACGCGCTCG GCTAATATGG CCTGCTGCAC  
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG  
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG  
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC  
 10751 CGCCCGTGTT GATGGTGTAA GTGCAGTTGG CCATAACGGA CCAGTTAACG  
 GCGGGCACA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC  
 10801 GTCTGGTGAC CCGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC  
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG  
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC  
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGSTCC ATGACCATAG  
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG  
 GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCGGT CGCATCCAC  
 10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA  
 CGGCCCGAG GCCCCCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT  
 11001 GATGTACCTG GACATCCAGG TGATCCGGC GGCGGTGGTG GAGGCGCGCG  
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC  
 11051 GAAAGTCGCG GACGCGGTTT CAGATGTTGC GCAGCGGCAA AAAGTGCTCC  
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG  
 11101 ATGGTCGGGA CGCTCTGGCC GGTGAGGCGC GCGCAATCGT TGACGCTCTA  
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CCGGTTAGCA ACTGCGAGAT  
 11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG  
 CTGGCACGTT TTCTCTCGG ACATTGCCCC GTGAGAAGGC ACCAGACCAC  
 11201 GATAAATTCT CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA  
 CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT  
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA  
 AGGCCGGCAG GCGGCACTAG GTACGCCAAT GCGGGGCGCA CAGCTTGGGT  
 11301 GGTGTGCGAC GTCAGACAAC GGGGAGTGC TCCTTTTGGC TTCCTTCCAG  
 CCACACGCTG CAGTCTGTTG CCCCCTCACG AGGAAAACCG AAGGAAGGTC

Figure 26L

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATT AAGTGGCTCGC TCCCTGTAGC  
TTCGCCAATC CGACCTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCGG GTTCGAGTCT  
GCCTCCCAAT AAAAGGTTC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501 CGGACCGGCC GGAATGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG  
GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTT

11551 ACCCCGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTGTGCTT  
TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601 TTCCAGATG CATCCGGTGC TGGCGCAGAT GCGCCCCCTT CCTCAGCAGC  
AAGGTCTAC GTAGGCCACG ACGCCGTCTA CCGGGGGGGA GGAGTCGTCG

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCTTC CCTCCTCTCT  
CCGTCTCTGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA  
TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGCGGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG  
AATGCTTGGG GCGCGCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC

11801 GCGAGGGCCT GCGCGGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG  
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT  
CACGTGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT  
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG  
AGGTGCGTCC CCGCGTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001 CCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG  
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCG ACCTGGTAAC CGCATAAGAG CAGACGGTGA  
GCGTGTGCAC CGCCGGCGGC TGGACCATTG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT  
TGGTCTCTA ATTGAAAGT TTTTCGAAAT TGTGTTGTCG CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT  
CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT  
TTCGCGCGAC CTCGTTTGG GTTTATCGTT CGGCGAGTAC CGCGTCGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTGAG GGATGCGCTG  
AGGAATATCA CGTCGTGTCG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG  
 GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTC

12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCGC  
 ACCGGCGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG

12451 AAGATATACC ATACCCCTTA CGTTCCTATA GACAAGGAGG TAAAGATCGA  
 TTCTATATGG TATGGGGAAT GCAAGGGTAT CTGTTCTCTC ATTTCTAGCT

12501 GGGGTTCTAC ATGCGCATGG CGCTGAAGST GCTTACCTTG AGCGACGACC  
 CCCCAAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGCTGCTGG

12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG  
 ACCCGCAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC

12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT  
 GCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTTCCCGGGA

12651 GGCTGGCACG GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG  
 CCGACCGTGC CCGTCGCGCG TATCTCTCCG GCTCAGGATG AAAGTGCGCC

12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG  
 CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC

12752 GCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG  
 CGGCTGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC

12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT  
 GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA

12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG  
 TGATTGCGCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCCTGGGCC

12901 CGGTGCGGGC GCGCTGCGAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAC  
 GCCACGCCCCG CCGCGACGTC TCGGTGCGCA GGCCGGAATT GAGGTGCTTG

12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC  
 CTGACCGCGG TCCAGTACCT GCGGTAGTAC AGCGACTGAC GCGCGTTAGG

13001 TGACGCGTTC CGGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG  
 ACTGCGCAAG GCCGTGCTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC

13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCACGAGAA GGTGCTGGCG  
 TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC

13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCG ACGAGGCCGG  
 TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC

13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA  
 GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTGCGCGT

13201 ACGTGCAGAC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCGTG  
 TGCACGTCTG GTTGGAACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

13301 ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG  
TGATTTGCCG AAGGACTCAT GTGTCGGGCG GTTGACGGC GCCCTGTCC

13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA  
TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG  
GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAAGTTGC  
ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTGAACG

13501 AGGGGCTGTG GGGGGTGCGG GCTCCCACAG GCGACCGCGC GACCGTGTCT  
TCCCCGACAC CCCCCACGCC CGAGGGTGTC CGCTGGCGCG CTGGCACAGA

13551 AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT  
TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA  
GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651 CACTGTACCG CGAGGCCATA GGTGAGGCGC ATGTGGACGA GCATACTTTC  
GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701 CAGGAGATTA CAAGTGTGAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG  
GTCCTCTAAT GTTCACAGTC GGCAGCGGAC CCCGTCCTCC TGTGCCCGTC

13751 CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC  
GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG  
GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC

13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCCAGCGT  
GTCGTCTCGC ACTCGGAATT GGAATACGCG CTGCCCCATT GCGGGTCGCA

13901 GCGGCTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA  
CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCATCG CGCGGCCGCG  
TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001 GTGAACCCCG AGTATTTTAC CAATGCCATC TTGAACCCCG ACTGGCTACC  
CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051 GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCAGG GGTAACGATG  
CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101 GATTCCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG  
CTAAGGAGAC CCGCTGTAT CTGCTGTGCG AAAAAAGGGG CGTTGGCGTC

14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA  
TGGGACGATC TCAACGTTGT CCGCTCGTC CGTCTCCGCC GCGACGCTTT

Figure 260

14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC  
GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GCGGAGGAGG AGTACCTAAA  
TCGTGAGCGT GGTGGGCGGG CCGGACGAC CCGCTCCTCC TCATGGATTT

14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTT  
GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAAG

14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG  
GGTTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG  
ATGCGCGTCC TCGTGTCCTT GCACGGTCCG GCGCGGGGCG GGTGGGCAGC

14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG  
AGTTTCCGTG CTGGCAGTCG CCCAGACCA CACCCTCCTG CTACTGAGCC

14551 CAGACGACAG CAGCGTCCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG  
GTCTGCTGTC GTCGCAGGAC CTAAACCCCT CCTCACCGTT GGGCAAACGC

14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAATAA AAAAGCATGA  
GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTCTT  
ACGTTTTATT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701 GTATTCCTCT TAGTATGCGG CCGCGGGCGA TGTATGAGGA AGGTCTCTCT  
CATAAGGGGA ATCATAACGC GCGCGCCGCT ACATACTCCT TCCAGGAGGA

14751 CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCCTGGG  
GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCAACGCC GCCGCGACCC

14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC  
AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG

14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC  
ACGCCGGATG GCGCCCTCTT TTGTCGTAGG CAATGAGACT CAACCGTGGG

14901 CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT  
GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCCTACA

14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA  
CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAAGAC TGGTGCCAGT

15001 TTCAAAACAA TGAATACAGC CCGGGGGAGG CAAGCACACA GACCATCAAT  
AAGTTTTGTT ACTGATGTCG GGCCCCCTCC GTTCGTGTGT CTGGTAGTTA

15051 CTTGACGACC GGTGCACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC  
GAACTGCTGG CCAGCGTGAC CCCGCCGCTG GACTTTTGGT AGGACGTATG

15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTAAAGGCGC  
GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCGCGC

Figure 26 P

15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA  
CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT  
ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG  
CTGGTATCTG GAATACTTGT TGCCTAGCA CCTCGTGATG AACTTTTACC

15301 GCAGACAGAA CGGGTTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC  
CGCTGTCTT GCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG  
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT  
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCTTA

15451 GCGGGGTGGA CTTACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC  
CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA  
TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAAC ATTCCCGCAC TGTGGATGT GGACGCCTAC CAGGCGAGCT  
CCCACCATG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GCGGGGGTG GCGCAGGCGG CAGCAACAGC  
ACTTCTACT GTGGCTTGTC CCGCCCCAC CGCGTCCGCC GTCGTTGTG

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA  
TCACCGTCGC CGCGCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701 GCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA  
CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC  
GTGCCCAGCT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801 GCCCCCGCTG CGCAACCCGA GGTGAGAAG CCTCAGAAGA AACC GGATGAT  
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA  
GTTTGGGGAC TGTCCTCTGT CGTTCTTTGC GTCAATGTTG GATTATTCTG

15901 ATGACAGCAC CTTACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC  
TACTGTCTG GAAGTGGGTG ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GGCGACCCCTC AGACCGGAAT CCGTCTATGG ACCCTGCTTT GCACTCCTGA  
CCGCTGGGAG TCTGGCCTTA GCGGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC  
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG  
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 Q

16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCAGCTGT  
CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA

16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC  
AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG

16251 ATCACCACCG TCAGTGAAAA CGTTCCTGCT CTCACAGATC ACGGGACGCT  
TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA

16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG  
TGGCGACGCG TTGTCGTAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC

16351 CCAGACGCGC CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG  
GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC

16401 CCGCGCGTCC TATCGAGCCG CACTTTTGA GCAAGCATGT CCATCCTTAT  
GGCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCGTACA GGTAGGAATA

16451 ATCGCCAGC AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT  
TAGCGGGTCG TTATTGTGTC CGACCCCGA CGCGAAGGGT TCGTTCTACA

16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG  
AACC GCCCG GTTCTTCGCG AGGCTGGTTG TGGGTCACGC GCACGCGCCC

16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC  
GTGATGGCGC GCGGGACCCC GCGCGTGTTT GCGCCGGCGT GACCCGCGT

16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA  
GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT

16651 CGCCACGCC GCCACAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG  
GCGGGTGCGG CGGTGGTCAC AGGTGTCACC TGCGCCGGTA AGTCTGGCAC

16701 GTGCGCGGAG CCCGGCGCTA TGCTAAATG AAGAGACGGC GGAGGCGCGT  
CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA

16751 AGCACGTGCG CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG  
TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGGCGGGTT GCGCGCCGCG

16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGGC GGCCATGCGG  
GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC

16851 GCCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG  
CGGCGAGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GTTCCAGGT

16901 GCGACGAGCG GCCGCCGAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG  
CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC

16951 GTCGACGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC  
CAGCGTCCCC GTTGACATA ACCCACGCGC TGAGCCAATC GCCGACGCG

17001 GTGCCCCTGC GCACCCGCC CCCGCGCAAC TAGATTGCAA GAAAAAATA  
CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT

Figure 26 R



17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG  
GATACAGGTT CGCGTTTATG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCGCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA  
CTCTAGATAC CGGGGGGCTT CTTCTTCTC GTCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG  
CGATTTCGCC CAGTTTTTCT TTTCTTTCT ACTACTACTA CTTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG  
TGCTCCACCT TGACGACGTG CGATGGCGCG GTTCCGCTGC CCATGTCACC

17301 AAAGGTGCAC GCGTAAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT  
TTTCAGCTG CGCATTTTGC ACAAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCCGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG  
ATGCGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG  
ACATGCCGCT GCTCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA  
AAACGGATGC CTTTCGCCGT ATTCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC  
CCCGTTGGGT TGTGGATCGG ATTTCGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT  
GGCGGAACG TGGCAGGCTT CTTTTCGCGC CGGATTTGCG GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA  
CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAATGA CCGTGAACC TGGGCTGGAG CCCGAGGTCC  
TCTACAGAAC CTTTTTACT GGCACCTTG ACCCGACCTC GGGCTCCAGG

17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG  
CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTGAGA TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA  
CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GCGGTTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCAGTTGC CTCAGCGGTG GCGGATGCCG  
CCCGTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCCTACGGC

17851 CGGTGCAGGC GGTGCTGCG GCCGCGTCCA AGACCTCTAC GGAGGTGCAA  
GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCAGCGCC CGCGCCGTTT  
TGCTTGGCA CCTACAAAGC GCAAAGTCGG GGGCCGCGG CGCGGCAAG

17951 GAGGAAGTAC GCGCGCCCA GCGCGCTACT GCCGAATAT GCCCTACATC  
CTCCTTCATG CCGCGGCGGT CCGCGGATGA CGGGCTTATA CGGGATGTAG

Figure 26S

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG  
 TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGGCGGCCGG

18101 TCGCCGTGCG CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC  
 AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCAGC  
 CGCTTCCTCC GTCCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTCC

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG  
 TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAG

18251 CCGCTTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA  
 GCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TCGCACCAC  
 CCGCGTACCG GCCGGTGCCG GACTGCCCGC CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCTT  
 GCCGCCGCCG CGCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT  
 GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG  
 GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC

18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC  
 TTTTATAGTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC  
 ATAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AAACCTGGCA GATATCGGCA CCAGCAATAT  
 CCGAGCGCGG GCAAGTACCC TTTGACCGT CTATAGCCGT GGTGCTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAATAATT  
 CTCGCCACCG CGGAAGTCGA CCCCGAGCGA CACCTCGCCG TAATTTTAA

18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGCA AGGCCTGGAA CAGCAGCACA  
 AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTCC AACAAAAGGT  
 CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC  
 CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTTGG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA  
 TCCGTACAGT TTTATTCTAA TTGTCATTCT AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GCGGTGGCGA  
 CTCTCGGAG GTGGCCGGCA CCTCTGTCAC AGAGGTCTCC CCGCACCGCT

Figure 26T

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT  
 TCGGAGGGAG CATGCTCCTC CGTGATTTTCG TTCCGGACGG GTGGTGGGCA  
 19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC  
 GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG  
 19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG  
 CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC  
 19151 GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC  
 CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG  
 19201 GCCGCCAGCG GTCCGCGATC GTTGCGGGCC GTAGCCAGTG GCAACTGGCA  
 CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT  
 19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC  
 TTCGTGTGAC TTGTCTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG  
 19301 GACGATGCTT CTGATAGCTA ACGTGTGCTA TGTGTGTCAT GTATGCGTCC  
 CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATA CGCAGG  
 19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA  
 TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT  
 19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC  
 ACCGATGGGG AAGCTACTAC GCGGTCACCA GAATGTACGT GTAGAGCCCG  
 19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCCGCG  
 GTCCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG  
 19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG  
 GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC  
 19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG  
 GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGCGACGCC  
 19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT  
 AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA  
 19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT  
 GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA  
 19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT  
 AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA  
 19751 GGCACGCTT ACAACGCCCT GGCTCCCAAG GGTGCCCAA ATCCTTGCGA  
 CCGTGACGGA TGTTCGCGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT  
 19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG  
 TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC  
 19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAA ACTCAC  
 TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTT GAGTG

Figure 26 U

19951 TCAAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTTC  
 AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAGG  
 20001 AACCTGAACC TCAAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT  
 TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTGT TCTTTAATTA  
 20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA  
 GTACGTCGAC CCTCTCAGGA TTTTTTCTGA TGGGGTTACT TTGGTACAAT  
 20101 CGGTTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG  
 GCCAAGTATA CGTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAAGAAG  
 20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTTC  
 ATTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG  
 20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT  
 AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTCG  
 20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT  
 CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA  
 20301 CTTACATGCC CACTATTAAG GAAGGTAAC CACGAGAACT AATGGGCCAA  
 GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTTGA TTACCCGGTT  
 20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTTAT  
 GTTAGATACG GGTGTCCGG ATTAATGTAA CGAAAATCCC TGTAAAATA  
 20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC  
 ACCAGATTAC ATAATGTTGT CGTGCCCAT ATACCCACAA GACCGCCCGG  
 20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG  
 TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTTCTGTC TTTGTGTCTC  
 20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT  
 GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA  
 20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA  
 AAGATACACC TTAGTCCGAC AACTGTGAT ACTAGGTCTA CAATCTTAAT  
 20601 TTGAAAATCA TGGAACTGAA GATGAACCTC CAAATTACTG CTTTCCACTG  
 AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC  
 20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAAACAGG  
 CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC  
 20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG  
 AGTCCTTTTA CCTACCCCTT TTCTACGATG TCTTAAAAGT CTATTTTAC  
 20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC  
 TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG  
 20801 CTGTGGAGAA ATTTCTGTG CTCCAACATA GCGCTGTATT TGCCCGACAA  
 GACACCTCTT TAAAGGACAT GAGGTGTAT CCGGACATAA ACGGGCTGTT

Figure 26 v

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC  
TGCTGATGTA CTTGTTTCGCT CACCACCGAG GGCCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCAGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC  
TAATTGGAAC CTCGTGCGAC CAGGGAAGTG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG  
TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT  
CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA  
CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC  
GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC  
ATTCCCAACT GCCTCGGTGCG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT  
TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AAGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA  
ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC  
TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTTCACCGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTCCCGCGGC TGGGCCTTCA CGCGCCTTAA  
GGGAGGGCGT TGACCCGCGG AAAGGCGCGG ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT  
CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCTTAC CTAGATGGAA CCTTTTACCT CAACCACACC  
TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA  
AAATTCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG  
ACTGGCGGAC GAATGGGGGT TGCTCAAACCT TTAATTGCGG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTTCCTG  
CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC  
CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA  
TCTCTCGATG TTCTTGGCGT ACATGAGGAA GAAATCTTTG AAGGTGCGGT

Figure 26 w

21851 GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC  
CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA  
GTGGTACGCG CTCCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT  
ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCAA AGAAACGCTA

22001 CGCACCCCTTT GCGGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC  
GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCCACGCGC  
TGAGTGCTCG GACCCGGTTT TGGAAAGAGT GCGGTTGAGG CGGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCAC CCTTCTTTAT  
ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG  
CAAAACAAAC TTCAGAAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTCTCGGCC GCAACGCCA  
GCAGTAGCTT TGGCACATGG ACGCGTGCGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA  
GTTGTATTTC TTCGTTGTT TAGTTGTTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGGTTG TGGGCCATAT  
CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA

22351 TTTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA  
AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC  
CGAGCGGACG CCGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGCATG

22451 ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT  
TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA  
CTCGGGAAAC CGAAAAGACT GGTGCTGAG TTCGTCCAAA TGGTCAAAC

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT  
CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC  
CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC  
CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCGG

22701 CCAAACCTCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC  
GGTTTGAGGG TACCTAGTGT TGGGGTGGTA CTTGGAATAA TGGCCCCATG

Figure 26 x

22801 CAGGAACAGC TCTACAGCTT CCTGGAGGCG CACTGCCCC ACTTCGCAAG  
GTCTTTGTCTG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGTCAC TTGAAAAACA  
GGTGTACAGC GTCTAATCCT CGCGGTGAAG AAAACAGTG AACTTTTGT

22901 TGTAATAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT  
ACATTTTAT TACATGATCT CTGTGAAAGT TATTCCGTT TACGAAATA

22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT  
AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA

23001 TTAATAATCA AAGGGTTCTT GCCGCGCATC GCTATGCGCC ACTGGCAGGG  
AATTTTATAGT TTCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC  
TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC  
TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151 CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC  
GTTGCGCAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC  
GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT  
TGATAGTCGC GGCCCAACCAC GTGCGACCGG TCGTGCAGAGA ACAGCCTCTA

23301 CAGATCCGCG TCCAGGTCTT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT  
GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC  
AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG  
AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451 ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT  
TATGTCGCGG ACGTATTTTC GGAACTAGAC GAATTTTCGG TGGACTCGGA

23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAAGTATTG  
AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTTGGAGAT  
CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA

23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG  
GACGTGGTGT AAAGCCGGGG TGCCCAAGAA GTGCTAGAAC CGGAACGATC

23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA  
TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26 Y

23701 ATCACGTGCT CCTTATTTAT CATAATGCTT CCGTGTAGAC ACTTAAGCTC  
TAGTGACGCA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT  
CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG  
GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG  
TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG  
GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC  
GAAGGTGAAC CAGTCCGTCA TCAAAC TTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA  
TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT  
GCGTCTGTGC TAGCCGTGTG AGTCGCCCCA GTAGTGGCAT TAAAGTGAAA

24101 CCGCTTCGCT GGGCTCTTCC TCTTCTCTT GCGTCCGCAT ACCACGCGCC  
GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGCGCGG

24151 ACTGGGTCGT CTTCAATCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC  
TGACCCAGCA GAAGTAAGTC GGCGGCGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTGTGCTGAA ACCCACCATT TGTAGCGCCA  
TACGAATAA TCGTGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG  
GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC  
GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA  
GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCCTC  
CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GGCGGCGACG GGGACGGGGA  
TAGGCGAAAA AACCCCGCGG GGCCCCCTCCG CCGCCGCTGC CCCTGCCCCCT

24501 CGACACGTCC TCCATGGTTG GGGGACGTG CGCCGCACCG CGTCCGCGCT  
GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCTTCTTCC  
GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC  
ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG



24701 CTACCACCTT CCCCCTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT  
 GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA  
  
 24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT  
 TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA  
  
 24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG  
 TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCCTCTC CGTTTGCTCC  
  
 24851 AACAAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA  
 TTGTTACAGC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT  
  
 24901 GACGACGTGC TGTTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA  
 CTGCTGCACG ACAACTTCGT AGACGTGCGG GTCACGCGGT AATAGACGCT  
  
 24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCTT CGCCATAGCG GATGTCAGCC  
 GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG  
  
 25001 TTGCCTACGA ACGCCACCTA TTCTACCGC GCGTACCCCG CAAACGCCAA  
 AACGGATGCT TGCGGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT  
  
 25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCGGTATT  
 CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA  
  
 25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACCTGCA  
 ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT  
  
 25151 AGATACCCCT ATCCTGCCGT GCCAACCGCA GCCGAGCGGA CAAGCAGCTG  
 TCTATGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTCGAC  
  
 25201 GCCTTGCGGC AGGGCGCTGT CATACCTGAT ATCGCCTCGC TCAACGAAGT  
 CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA  
  
 25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG  
 CGGTTTTTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC  
  
 25301 CTCTGCAACA GGAAACAGC GAAAATGAAA GTCACCTCTG AGTGTGGTG  
 GAGACGTTGT CCTTTTGTCG CTTTACTTT CAGTGAGACC TCACAACCAC  
  
 25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAC GCAGCATCGA  
 CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT  
  
 25401 GGTCACCCAC TTTGCCTACC CGGCACCTAA CCTACCCCGC AAGGTCATGA  
 CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT  
  
 25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG  
 CGTGTCACTA CTCACTCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC  
  
 25501 GATGCAAAAT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA  
 CTACGTTTAA ACGTTCTTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT  
  
 25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG  
 GCTCGTCGAT CGCGCGACCG AAGTTTGGCG GCTCGGACGG CTGAACCTCC

Figure 26 AA

25651 TGCATGCAGC GGTTCCTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA  
 ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTCGCGT TCGATCTCCT  
 25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA  
 TTGTAACGTG ATGTGGAAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT  
 25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC  
 AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAAACGTG  
 25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC  
 CTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCCTCCG  
 25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT  
 CGCGGCGCTG ATGCAGGCGC TGACGCAAAT GAATAAAGAT ACGATGTGGA  
 25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC  
 CCGTCTGCCG GTACCCGCAA ACCGTGCTCA CGAACCTCCT CACGTTGGAG  
 25951 AAGGAGCTGC AGAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC  
 TTCCTCGACG TCTTTGACGA TTTCGTTTTG AACTTCCTGG ATACCTGCCG  
 26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GCGGACATC ATTTTCCCCG  
 GAAGTTGCTC GCGAGGCACC GCGCGTGGA CCGCCTGTAG TAAAAGGGGC  
 26051 AACGCCTGCT TAAAACCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA  
 TTGCGGACGA ATTTTGGGAC GTTGTCCAG ACGGTCTGAA GTGGTCAGTT  
 26101 AGCATGTTGC AGAACTTTAG GAACCTTATC CTAGAGCGCT CAGGAATCTT  
 TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCGA GTCCTTAGAA  
 26151 GCCCCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC  
 CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCTATG  
 26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC  
 CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG  
 26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG  
 TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC  
 26301 TCTACTGGAG TGTCACGTGC GCTGCAACCT ATGCACCCCG CACCGCTCCC  
 AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG  
 26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT  
 ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA  
 26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTGAA  
 CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCAACTT  
 26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG  
 TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC  
 26501 AGGACTACCA CGCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCG  
 TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG AGCTTACCGC CTGCGTCATT ACCCAGGGCC ACATTCCTGG  
 GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCC GG TGTAAGAACC  
 26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG  
 GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC  
 26651 GACGGGGGGT TTAATTGGAC CCCCAGTCCG GCGAGGAGCT CAACCCCAATC  
 CTGCCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG  
 26701 CCCCCGCCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA  
 GGGGGCGGCG GCGTCGGGAT AGTCGTCGTC GCGCCCCGGG AACGAAGGGT  
 26751 GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG  
 CCTACCGTGG GTTTTCTTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC  
 26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA  
 CTCCTTATGA CCCTGTCACT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT  
 26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTCTG  
 CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC  
 26901 AAGAGGTGTC AGACGAAACA CCGTCACCCT CCGTCGCATT CCCCTCGCCG  
 TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC  
 26951 GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC  
 CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG  
 27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA  
 AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTTGGCA TCTACCCTGT  
 27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA  
 GGTGACCTTG GTCCCGGCCA TTCAGGTTCTG TCGGCGGCGG CAATCGGGTT  
 27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC  
 CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG GTTCTTTGCG  
 27151 CATAGTTGCT TGCTTGCAAG ACTGTGGGGG CAACATCTCC TTCGCCGCC  
 GTATCAACGA ACGAACGTTT TGACACCCCC GTTGTAGAGG AAGCGGGCGG  
 27201 GCTTTCTTCT CTACCATCAC GCGGTGGCCT TCCCCGTAA CATCCTGCAT  
 CGAAAGAAGA GATGGTAGTG CCGCACCGGA AGGGGGCATT GTAGGACGTA  
 27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA  
 ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT  
 27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA  
 GTCGTCGCCG GTGTGTCTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT  
 27351 AAGCCCAAGA AATCCACAGC GCGGGCAGCA GCAGGAGGAG GAGCGCTGCG  
 TTCGGGTTCT TTAGGTGTCT CCGCCGTCGT CGTCCTCCTC CTCGCGACGC  
 27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT  
 AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCTTAAA  
 27451 TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG  
 AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTCTC

Figure 26: AC

27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC  
 AGTGTTTTCG CTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT  
 AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC  
 AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG

27701 GCCAGCACCT GTTGTGAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT  
 CGGTCGTGGA CAACAGTCGC GGTAATACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA  
 TGTACACCTC AATGGTCGGT GTTTACCTTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC  
 CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG  
 GGCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC  
 GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC  
 CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG  
 GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTGCG CCGGGCAGGG TATAACTCAC  
 GCGGCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC  
 GACTGTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GCGCCCGGCC  
 GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC  
 CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAACT CTGCAATTTA TTGAGGAGTT  
 AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC  
 ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GCGGGACGGC  
 GCCTAGTTAA ATAAGGATTG AACTGCGCC ATTTCTGAG CCGCCTGCCG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT  
 ATGCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT  
CCAGGTGACA GCGGCGGTGT TCACGAAACG GGCCTGAGG CCACTCAAAA

28501 GCTACTTTGA ATTGCCCAG GATCATATCG AGGGCCCGGC GCACGGCGTC  
CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG

28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC  
GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG  
GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651 TGATTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT  
ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAAACGGTA

28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT  
GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

28751 ATCGCCATCC TGTAACGCC ACCGTCTTCA CCCGCCAAG CAAACCAAGG  
TAGCGGTAGG ACATTTGCGG TGGCAGAAAT GGGCGGGTTC GTTTGGTTCC

28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG  
GCTTGGAATG GACCATGAAA ATTGTAAGAGA GGGAGACACT AAATGTTGTC

28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT  
AAAGTTGGGT CTGCCCTACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGA ACGTACGAGT  
TGAGGTAGTC TTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951 GCGTCACCG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT  
CGCAGTGGCC GGCAGCTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT  
AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA

29051 AGAAAACCTT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT  
TCTTTTGGGA ATCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA  
CTTGTTAAGT TCGTTGAGAT GCGCGATAAG ATTAAGTCCA AAGAGATCTT

29151 TCGGGGTTGG GGTATTCTC TGTCTTGTGA TTCTCTTTAT TCTTATACTA  
AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT

29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA  
TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251 TTGTCAGCTT TTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT  
AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA

29301 AATCCTAGGT TTA CTACCC TTGCGTCAGC CCACGGTACC ACCCAAAGG  
TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC

29351 TGGATTTTAA GGAGCCAGCC TGTAATGTGA CATTGCGAGC TGAAGCTAAT  
ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTTTATGCT ATTTGGCAGC  
AGCGGTGTTT TTGTTTAAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT  
GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT  
TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA  
CATGTACTCG TTTGTTCATAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTC  
TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTTATGA  
CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTACTAAGTT ACAAAGCTAA TGTCACCACT  
CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA  
TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCC GGTCAT TTCCTGCTCA ATACCATTCC  
TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA  
GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC  
CAGTCCGAAG GACCTACAGT CGTAGACTGA AACC GGTCGT GGACAGGGCG

30001 GGATTTGTTT CAGTCCAAC TACAGCGACCC ACCCTAACAG AGATGACCAA  
CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCGG CTACCGGACT TACATCTACC ACAAATACAC  
GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101 CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG  
GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG  
AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG  
GACGGATTTC GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC  
ACGATGTGGG TTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT  
TACAAGAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30401 TGCGGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT  
ACGCCAAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA

30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG  
ACGAAATGCC TAAACAGTGG GAGTGCGAGT AGACGTCGGA GTAGTGACAC

30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA  
CAGTAGCGGA AATAGGTCAC GTAAGTGACC CAGACACACG CGAAACGTAT

30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA  
AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT

30601 GAATTCTTTA ATTATGAAAT TTAAGTGAC TTTTCTGCTG ATTATTTGCA  
CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT

30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA  
GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT

30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG  
ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC

30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTTC  
GCTAGAAAGG CTTCGGACCA ATATACGTTA GTAGAGACAA TACCACAAGA

30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG  
CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAC GTAAACCGACC

30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT  
TTGCGTTATC TACGGTACTT GGTGGGTGA AAGGGGCGCG GCGGATACGA

30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCAGCC AATCAGCCTC  
AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG

30951 GCCCACCTTC TCCCACCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA  
CGGGTGGAAG AGGGTGGGG TGACTTTAGT CGATGAAATT AGATTGCTCT

31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC  
CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG

31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT  
TCGCGGACGA TCTTCTGCG TCCCGTCGCC GGCTCGTTGT CCGGTACTTA

31101 CAAGAGCTCC AAGACATGGT TAAGTTGCAC CAGTGCAAAA GGGGTATCTT  
GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA

31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC  
AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCAATTA TGGTGGCCTG

31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG  
TGGCGGAATC GATGTTCAAC GGTGGGTTTC CAGTCTTTAA CCACCAAGTAC

31251 GTGGGAGAAA AGCCCATTAC CATAACTCAG CACTCGGTAG AAACCGAAGG  
CACCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA  
TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

31401 ATAATAAAGC ATCACTTACT TAAATCAGT TAGCAAATTT CTGTCCAGTT  
TATTATTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC  
ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC  
GAGGACCGAC GTTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC  
GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG  
CGCGTTCTGG CAGACTTCTA TGGAAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAACGTG GCCTTTTCTT ACTCCTCCCT TTGTATCCCC  
CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG  
GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC  
TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTTA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAATG TAACCACTGT  
GAGAGAGACC TGCTCCGGCC GTTGAATGG AGGGTTTTAC ATTGGTGACA

31851 GAGCCACCT CTCAAAAAA CCAAGTCAA CATAAACCTG GAAATATCTG  
CTCGGGTGA GAGTTTTTT GTTCAGTTT GTATTGGAC CTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT  
GTGGGGAGTG TCAATGGAGT CTTGCGGATT GACACCGACG GCGGCGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCCTAAC  
GATTACCAGC GCGGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG

32001 CGTGACGAC TCCAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT  
GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTCACA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT  
GTCTTCCTTT CGATCGGGAC GPTGTAGTC CGGGGGAGTG GTGGTGCTA

32101 AGCAGTACCC TTACTATCAC TGCCTCACC CCTCTAACTA CTGCCACTGG  
TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC  
ATCGAACCCG TAACTGAACT TTCTCGGGTA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CCTTTCATG TAACAGACGA CCTAAACACT  
ATCTGATTT CATGCCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH



32301 AACTAAAGTT ACTGGAGCCT TGGGTTTGA TTCACAAGGC AATATGCAAC  
TTGATTTCAA TGACCTCGGA ACCCAAACCT AAGTGTTCGG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA  
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTGTGTC TGCGBAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCACCTAA ATCTAAGACT  
GAATACAAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTTA TAACTCAGC CCACAACCTG GATATTAACT  
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT  
TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA

32551 GAGGTAAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT  
CTCCAATTGG ATTCGTGACG GTTCCCAAC TACAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA  
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGGA TTACGTGGTT

32651 ACACAAATCC CCTCAAAACA AAAATGGGCC ATGGCCTAGA ATTTGATTCA  
TGTGTTTAGG GGAGTTTGT TTTTAACCGG TACCGGATCT TAACTAAGT

32701 AACAAGGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC  
TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCGTG

32751 AGGTGCCATT ACAGTAGGAA ACAAAAATAA TGATAAGCTA ACTTTGTGGA  
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT  
GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAATCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT  
TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTTG GCTGTTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC  
AAGTCAAAAC CGACAATTTT CGTCAAACCG AGGTTATAGA CTTGTCAAG

32951 AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC  
TTTACAGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTG

33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC  
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG  
ACTTCCGTGT CGGATATGTT TGCGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTCAGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA  
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCAATTGA ACAGTCAGTT

33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT  
CAATGAATT TGCTCTGTT TTGATTTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33251 CATTTTCATG GGA CTGGTCT GGC CACA ACT ACATTAATGA AATATTTGCC  
GTAAAAGTAC CTGACCAGA CCGGTGTTGA TGTAAATTACT TTATAAACGG

33301 ACATCCTCTT ACAC TTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG  
TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC

33351 TGT TATGTTT CAACGTGTTT ATTTTTC AAT TGCAGAAAAT TTCAAGTCAT  
ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC  
AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAACTCACA GAACCTAGT ATTCAACCTG CCACCTCCCT  
CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC  
GGGTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT  
TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTCGA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA  
AAGGACAGCT CGGTTGCGA GTAGTACTA TAATTATTTG AGGGGCCCGT

33651 GCTCACTTAA GTTCATGTG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT  
CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT  
GGTTGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TCGCGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA  
CCCCATCTC AGTATTAGCA CGTAGTCTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AAAC TGCTGC CGCCGCCGCT CCGTCTGCA GGAATACAAC  
CGCGCGCTTA TTTGACGACG GCGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTGCG ACCGCCGCA GCATAAGGCG  
TACCGTCACC AGAGGAGTCG CTAATAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCTT GATCTCACTT AAATCAGCAC  
GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG  
TCATTGACGT CGTGTCTGG TGTATAACA AGTTTATAGG TGTCACGTTT

34001 GCGCTGTATC CAAAGCTCAT GGC GGGGACC ACAGAACCCA CGTGGCCATC  
CGCGACATAG GTTTCGAGTA CCGCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG  
TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC  
TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34201 GCTGGCCAAA ACCTGCCCCG CGGCTATACA CTGCAGGGAA CCGGGACTGG  
 CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC  
 34251 AACAA TGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC  
 TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG  
 34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT  
 CAGTACTATA GTTACAACCG TGTGTGTGCC GTGTGCACGT ATGTGAAGGA  
 34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC  
 GTCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTTGGG  
 34401 ATTCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA  
 TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT  
 34451 CTCACGTTGT GCATTGTCAA AGTGTACAT TCGGGCAGCA GCGGATGATC  
 GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG  
 34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC  
 GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCTCCA TCTGCTAGGG  
 34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTGG TCGTAGTGTC  
 ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG  
 34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCTGAAG CAAAACCAGG  
 TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC  
 34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC  
 ACGCCCGCAC TGTTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG  
 34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC  
 AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG  
 34751 CCTGGCTTCG GGTTCATGT AAATCCTTC ATGCGCCGCT GCCCTGATAA  
 GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT  
 34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCGTTT  
 GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTGGATG TGTAAGCAAG  
 34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT  
 ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA  
 34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG  
 AAAAAATAAG GTTTTCTAAT AGGTTTGGGA GTTTTACTTC TAGATAATTC  
 34951 TGAACGCGCT CCCCTCCGGT GCGGTGGTCA AACTCTACAG CCAAAGAACA  
 ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTTCTTGT  
 35001 GATAATGGCA TTTGTAAGAT GTTGACAAAT GGCTTCCAAA AGGCAAACGG  
 CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC  
 35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC  
 GGGAGTGCAG GTTCACCTGC ATTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26 AK

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA  
GGTGGAAGAG TTATATAGAG ATTCTGTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA  
AACATTTTTA GACGAGGTCT CCGGGGAGGT GGAAGTCGGA GTTCGTCTCGT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA  
TAGTACTAAC GTTTTTAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC  
TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC  
GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC  
GCGGTCCTTG GTACTGTTTT CTGGGGTGTG ACTAATACTG TGCCTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG  
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA  
GCTATATTTT ACGTTCCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC  
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTCC

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC  
AGGCCTTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTAAACATT  
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA  
TCTTCGGACA GAATGTTGTC CTTTTTGTTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA  
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTCATGTCC GGAGTCATAA TGTAAGACTC  
TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA  
CCATTTGTGT AGTCCAACATA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC  
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC  
GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTGT GTATTTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA  
ACTTTTGGG AGGACGGATC CGTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36101 AAAGAAAACC TATTAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA  
 TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT  
 36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA  
 CAGTGTACACA TTTTTCCTCG GTTCACGTCT CGCTCATATA TATCCTGATT  
 36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG  
 TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC  
 36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCACAAAC TTCCTCAAAT  
 GCTTGGATGC GGGTCTTTGC TTTCGGTTTT TTGGGTGTG AAGGAGTTTA  
 36301 CGTCACTTCC GTTTTCCAC GTTACGTCAC TTCCCATTTT AAGAAAATA  
 GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT  
 36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAAACCTA CGTCACCCGC  
 GTTAAGGGTT GTGTATGTTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG  
 36401 CCCGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA  
 GGGCAAGGGT GCGGGGCGCG GTGCAGTGT TGAGGTGGGG GAGTAATAGT  
 PacI  
 -----  
 36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA  
 ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT  
 36501 ATTCCGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC  
 TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG  
 36551 TCGCTTCCGG CGGCATCGGG ATGCCCGCGT TGCAGGCCAT GCTGTCCAGG  
 AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC  
 36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG  
 GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC  
 36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC  
 CTTGGCATT TTTCCGGCGA ACGACCGCAA AAAGGTATCC GAGGCGGGGG  
 36701 CTGACGAGCA TCACAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG  
 GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC  
 36751 ACAGGACTAT AAAGATACCA GGCCTTTCCC CCTGGAAGCT CCTTCGTGCG  
 TGCTCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC  
 36801 CTCTCCTGTT CCGACCTGCG CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
 GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG  
 36851 CTTCCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT  
 GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA  
 36901 TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT  
 AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT  
 GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA  
 37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC  
 TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAAGT TCACCACCGG  
 37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
 ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT  
 37151 AGCCAGTTAC CTTTCGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA  
 TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAAGTAG GCCGTTTGT  
 37201 ACCACCGCTG GTAGCGGTGG TTTTGTGTT TGCAAGCAGC AGATTACGGC  
 TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCTGTCG TCTAATGCGC  
 37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
 GTCTTTTCTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC  
 37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA  
 TCGGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT  
 37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT  
 AGTTTTTCCT AGAAGTGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA  
 37401 GAGTAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT  
 CTCATTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA  
 37451 CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG  
 GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC  
 37501 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA  
 ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT  
 37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA  
 TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT  
 37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG  
 GGTGCGTCGG CTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC  
 37651 CCTCCATCCA GTCTATTAAT TGTGCCGGG AAGCTAGAGT AAGTAGTTCG  
 GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC  
 37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT  
 GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA  
 37751 GTCACGCTCG TCGTTTGGA TGGCTTCATT CAGCTCCGGT TCCCAACGAT  
 CAGTGCAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA  
 37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC  
 GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG  
 37851 TTCGGTCTC CGATCGTTGT CAGAAGTAAG TTGGCCGAG TGTATCACT  
 AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26 AN

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG  
CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC  
ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT  
GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC CAGTTCGATG  
GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG  
ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA  
GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT

38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT  
ATCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA  
ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA  
TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT  
TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA  
TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

PacI

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38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)  
AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 A0

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1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   TAGTAGTGA TTATATGGAA TAAAACCTAA CTTGCGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGCG

651 CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

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*Figure 27A*



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901  TCGCTATTAC CATGGTGATG CGGTTTGGC AGTACATCAA TGGGCGTGGA
    AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951  TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
    ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
    ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
    TGTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
    CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
    GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
    AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCCGCT
    CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
    CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA
    CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
    GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
    GGCTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
    CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCAC TTCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
    GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC
    GGGTCTTCTC CGTCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCC
    ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCCTGGAG CCCGAGAAGG
    GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC
    ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGGCGGGT GGGGTACAGG

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Figure 27B

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1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT
      GAGGTTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG
      TGTTCCTGAC GATTTCTGGG CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCCCTC CCCCCTGCCT TCCTTGACCC TGGGAAGGTGC
      GGTAGACAAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC
      GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
      ACTCATCCAC AGTAAGATAA GACCCCCCAC CCCACCCCGT CCTGTCGTTC

2101 GGGGAGGATT GGGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC
      CCCCTCCTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT
      ATACCGGCTA GCCGCGCGGC ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTGTG ATCTGTTTTG
      CCCTTTCCTA TATATTCCAC CCCCAGAATA CATCAAAACA TAGACAAAAC

2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTGTC
      GTCGTCGGCG GCGGCGGTAC TCGTGTTGA GCAAACCTACC TTCGTAACAC

2301 A3CTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TGCCTCAGAA
      TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCCTGCCC GCAAACCTCTA
      ACACIACCCG AGGTCGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC
      GATGGAACCT GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCCG CTTAGCCGCG TGCAGCCACC GCCCGCGGGA TTGTGACTGA
      AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCGCG TTGCAAACAG TGCAGCTTCC CGTTCATCCG
      GAAACGAAAG GACTCGGGCG AACGTTTGTC ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC
      GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAAACTGG

2601 CGGGAACCTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT
      GCCCTTGAAT TACAGCAAAG AGTCGTGCAC AACCTAGACG CGGTGCTCCA

2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TCGGGTTTAA AACATAAATA
      AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT
      TTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

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Figure 27C

2751 TATTTAGGGG TTTTGC GCGG TAGGCC CGGGACCAGC GGTCTCGGTC  
ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGACTCTGGA  
CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTC CACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC  
ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA  
ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG  
CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT  
GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTLAGGTT  
CCCACGTATG CCCCCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA

3101 GGCTATGTTT CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA  
CCGATACAAG GGTCGGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCTAG TAGCTTAGAA  
GGTCGTGTCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC  
CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCT TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG  
GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCGC CGCCGGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA  
GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCCTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG  
AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA  
ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCACGCG TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG  
AAAGGGTGCG AAACCTCAAGT CTACCCCCCT AGTACAGATG GACGCCCCCG

3501 ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG  
TACTTCTTTT GCCAAAGGCC CCATCCCCCTC TAGTCGACCC TTCTTTCTCT

3551 GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC  
CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTCATCC  
GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTT  
GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGAAGTAC CGTACAAAAG

3701 CCTGACCAAA TCCGCCAGAA GGCCTCGCC GCCCAGCGAT AGCAGTTCTT  
GGACTGGTTT AGGCGGTCTT CCGCAGCGG CGGGTCGCTA TCGTCAAGAA

3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG  
CGTTCCCTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC

3801 CTTTTGAGCG TTTGACCAAG CAGTTCAGG CGGTCCCACA GCTCGGTCAC  
GAAAACTCGC AAACCTGGTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG  
GACGAGATGC CGTAGAGCTA GGTCGTATAG AGGAGCAAAG CGCCCAACCC

3901 GCGGCTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG  
CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC

3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG  
AGTACAGAAA GGTGCCCCGG TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT  
CACTTCCCCA CGCGAGGCCG GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051 GGTCTGTCTG GTGCTGAAGC GGTGCCGGTC TTCGCCCTGC GCGTCGGCCA  
CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101 GGTCATCTTT GACCATGGTG TCATAGTCCA GCCCTCCGC GCGTGGCCC  
CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG

4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG  
AACC GCCTG CGAACGGGAA CCTCCTCCGC GCGGTGCTCC CCGTCACGTC

4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT  
TGAAAACCTC CGCATCTCGA ACCGCGCTC TTTATGGCTA AGCCCCCTCA

4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG  
TCCGTAGGCG CGGCGTCCGG GCGGTCTGCC AGAGCGTAAG GTGCTCGGTG

4301 GTGAGCTCTG GCCGTTCCGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT  
CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351 GATGCGTTTC TTACCTCTGG TTTCATGAG CCGGTGTCCA CGCTCGGTGA  
CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCTG  
GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCTC GGACAGGAGC

4451 AGCGGTGTTT CGCGGTCTCT CTCGTATAGA AACTCGGACC ACTCTGAGAC  
TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC  
TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTACAC CTCCCCATCG

4551 GGTCGTGTGC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG  
CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCACACAC TTCTGTGTAC

4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC  
AGCGGGAGAA GCCGTAGTTC CTTCCTACTA CCAAACATCC ACATCCGGTG

Figure 27E

4701 CGTCCTCACT CTCTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT  
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCAGT  
CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTAC CTGGCCCGCG GTGATGCCTT  
AAGGTTTTTG CTCCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA  
ACTCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGCGGAT  
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTTT TGTCCGATC GCGCGCTCC TTGGCCGCGA  
CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACCGGCGCT

5001 TGTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTG GGGAAAGACG  
ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTTCTGC

5051 GTGGTGCCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGTCAG  
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG  
CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT  
AGGTCGTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCCAGA

5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCGGGCAG  
TCGACGCAGA GCAGGCCCCC CAGACGCAGG TGCCATTCTT GGGGCCCCGT

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT  
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTC AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA  
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC  
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC  
CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA  
AAGGTGGCGC CTACGACCGC GCGTGCATTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA  
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGACGCT  
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTCGCA

Figure 27F

5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC  
 CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG  
 5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT  
 CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA  
 5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAAACTC TTCGCGGTCT  
 CAGGGAAAAA AAAGGTGTCG AGCGCCAACT CCTGTTTGAG AAGCGCCAGA  
 5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC  
 AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG  
 5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GCGCGAGCAT CCCTTTTCTA  
 ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT  
 5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC  
 GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG  
 5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT  
 CGTTTCCACA GGGACTGGTA CTGAAACTCC ATGACCATAA ACTTCAGTCA  
 6001 GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGG  
 CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC  
 6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC  
 TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG  
 6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA  
 CGCGCTCCGT ATTTCAACGC AACTACGCC TTCCAGGGC CGTGGAGCCT  
 6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA  
 TGCCAAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT  
 6201 TGTTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG  
 ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAAGTAC  
 6251 GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG  
 CTTCCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC  
 6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA  
 GGGCAGGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTGCTGCT  
 6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG  
 TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC  
 6401 GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA  
 CAGGATTGTA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT  
 6451 GGTAAGCGGG TCTTGTTCCT AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT  
 CCATTCGCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA  
 6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACTT CATGACCAGC  
 GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTGC

Figure 27G

6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG  
ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC

6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG  
CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTACCCGA TAACTACACC

6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA  
ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT

6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA  
TTTTGCACGC GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT

6801 GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC  
CCAACTGGAC TGCTGGCGCG GTTTCCTTCG TCTCACCCTT AAATCGGGG

6851 TCGCCTGGCG GGTTCGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG  
AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC

6901 ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC  
TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGGCGCG

6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTCGGAG CTTGATGACA  
CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT

7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG  
TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC

7051 GTCAGGCGGG AGCTCCTGCA GGTTCACCTC GCATAGACGG GTCAGGGCGC  
CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG

7101 GGGCTAGATC CAGGTGATAC CTAATTTCCA GGGGCTGGTT GGTGGCGGCG  
CCCAGTCTAG GTCCACTATG GATTAAAGGT CCCCACCAA CCACCGCCGC

7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GCGCGACTA CGGTACCGCG  
AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC

7201 CGGCGGGCGG TGGGCCGCGG GGGTGTCTTT GGATGATGCA TCTAAAAGCG  
GCCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC

7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA  
CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCCT GGGCGGCCCT

7301 GAGGGGGCAG GGGCACGTCG GCGCCGCGCG CGGGCAGGAG CTGGTGCTGC  
CTCCCCGTC CCCGTGCAGC CGCGGCGCGC GCCCGTCTC GACCACGACG

7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTGTA TCTCCTGAAT  
CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA

7401 CTGGCGCCTC TGCGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG  
GACCGCGGAG ACGCACTTCT GCTGCCCCGG CCACTCGAAC TTGGACTTTC

7451 AGAGTTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAAA  
TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCGC GACCGCGTTT

Figure 27H

7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG  
 GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC  
 7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG  
 ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC  
 7651 AGGCCTCCCT CGTTCCAGAC GCGGCTGTAG ACCACGCCCC CTTCGGCATC  
 TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG  
 7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA  
 CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT  
 7751 AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG  
 TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC  
 7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTG  
 CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG  
 7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA  
 CAACTATAGG GGGTTCCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT  
 7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC  
 GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG  
 7951 TCCAGAAGAC GGATGAGCTC GCGGACAGTG TCGCGCACCT CGCGCTCAA  
 AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGGA GCGCGAGTTT  
 8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT  
 CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTTCCGGA  
 8051 CCCCTTCTTC TTCTTCTGGC GCGGTTGGGG GAGGGGGGAC ACGGCGGCGA  
 GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCGCCGCT  
 8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG  
 GGTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC  
 8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTTCTCG CGGGGGCGCA  
 CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCGT  
 8201 GTTGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG  
 CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCGAC  
 8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT  
 GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA  
 8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG  
 TCCATGAGGC GGCGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC  
 8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG  
 TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC  
 8401 AGCACCGTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA  
 TCGTGCCACC GCGCGCCGTC GCGCGCCGCC AGCCCCAACA AAGACCGCCT

Figure 27I



8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCCG  
AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGCC

8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA  
AGCCGGTACG GGGTCCGAAG CAAAACGTGA GCCGCGTCCA GAAACATCAT

8601 GTCTTGCAATG AGCCTTTCTA CCGGCACCTC TTCTTCTCCT TCCTCTTGTC  
CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG

8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG  
GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC

8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG  
ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC

8751 AAGCAGGGCT AGGTCGGCGA CAACGCGCTC GGCTAATATG GCCTGCTGCA  
TTCGTCCCGA TCCAGCCGCT GTTGCGCGAG CCGATTATAC CGGACGACGT

8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT  
GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA

8851 GCGCCCGTGT TGATGGTGTA AGTGCAGTTG GCCATAACGG ACCAGTTAAC  
CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG

8901 GSTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG  
CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC

8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT  
GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA

9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT  
GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA

9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT  
CCGGCCCCGA GGCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA

9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC  
TCTACATGGA CCTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG

9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC  
CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG

9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT  
GTACCAGCCC TGCAGAGCCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA

9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT  
TCTGGCACGT TTTCTCTCG GACATTGCGC CGTGAGAAGG CACCAGACCA

9301 GGATAAATTC GCAAGGGTAT CATGCGGAC GACCGGGGTT CGAGCCCCGT  
CCTATTAAAG CGTTCCATA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA

9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCCGG TGTCGAACCC  
TAGGCCGGCA GGCGGCACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

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9451  GCGCGGGCGG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG
      CCGCGCCGCC GACGACGCGA TCGAAAAAAC CGGTGACCGG CGCGCGTCGC

9501  TAAGCGGTTA GGCTGGAAG CGAAAGCATT AAGTGGCTCG CTCCCTGTAG
      ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC

9551  CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GGTTCGAGTC
      GGCTTCCCAA TAAAAGGTTT CCAACTCAGC GCCCTGGGGG CCAAGCTCAG

9601  TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA
      AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT

9651  GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTGTCT
      CTGGGGCGAA CGTTTAAGGA GGCCTTTGTC CCTGCTCGGG GAAAAACGA

9701  TTTCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG
      AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC

9751  CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCCT CCCCTCCTCC
      GCCGTTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG

9801  TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG
      ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC

9851  ATTACGAACC CCGCGGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG
      TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC

9901  GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG
      CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTC

9951  GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC
      CCACGTCGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG

10001 TGTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG
      ACAAGCGCT GCGCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC

10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT
      AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA

10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCGGGATT AGTCCCGCGC
      CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG

10151 GCGCACACGT GCGGGCCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG
      CGCGTGTGCA CCGCGGGCGG CTGGACCATT GGCGTATGCT CGTCTGCCAC

10201 AACCAGGAGA TTAACCTTCA AAAAAGCTTT AACAAACCAG TGCGTACGCT
      TTGGTCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA

10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG
      ACACCGCGCG CTCCTCCACC GATATCTGA CTACGTAGAC ACCCTGAAAC

10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GCGCGAGCTG
      ATTCGCGCGA CCTCGTTTTG GGTTTATCGT TCGGCGAGTA CCGCGTCGAC

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Figure 27K

10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA  
CGATTTGTAT CATCTCGGGC TCCC GGCGAC CGACGAGCTA AACTATTTGT

10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG  
AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC

10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCC  
CACC GGCGGT AGTTGATAAG GTACGAATCG GACCCGTTC AATGCGGGC

10551 CAAGATATAC CATACCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG  
GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCCCTC CATTTCTAGC

10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC  
TCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG

10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG  
GACCCGCAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC

10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC  
CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTCCCCGGG

10751 TGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG  
ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC

10801 GCGGCTGACC TGCGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG  
CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC

10851 GGCCGGACCT GGGCTGGCGG TGGCACC CGCGCTGGC AACGTCGGCG  
CCGCGCTGGA CCGGACCGCC ACCGTGGGCG CGCGCGACCG TTGCAGCCG

10901 GCGTGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG  
CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC

10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG  
ATGATTCGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC

11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA  
CGCCACGCCC GCGCGACGCT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT

11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC  
GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG

11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG  
GACTGCGCAA GGCCGTCTGTC GCGCTCCGGT TGGCCGAGAG GCGTTAAGAC

11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC  
CTTCGCCACC AGGGCCGCGC GCGTTTGGGG TGCTGCTCT TCCACGACCG

11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG  
CTAGCATTG CGCGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC

11251 GCCTGGTCTA CGACGCGCTG CTTAGCGCG TGGCTCGTTA CAACAGCGGC  
CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCCG

Figure 27L

11351 GGC GCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG  
CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC

11401 CACTAAACGC CTCCTGAGT ACACAGCCCG CCAACGTGCC GCGGGGACAG  
GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTGTCACGG CGCCCTGTCTC

11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGA CTGAGAC  
CTCCTGATGT GGTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG

11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA  
TGGCGTTTCA CTCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT

11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAACTTG  
CATCTGTTCC GGACGTCTGG CATTTGGACT CGGTCCGAAA GTTTTGAAC

11601 CAGGGGCTGT GGGGGGTGCG GGCTCCACA GGCAGCCGCG CGACCGTGTCTC  
GTCCCCGACA CCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG

11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT  
ATCGAACGAC TCGGGGTGA GCGCGGACAA CGACGACGAT TATCGCGGGA

11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACTAGG TCACTTGCTG  
AGTGCTGTC ACCGTCGCAC AGGGCCCTGT GTATGGATCC AGTGAACGAC

11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT  
TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA

11801 CCAGGAGATT ACAAGTGTCA GCCGCGCGCT GGGGCAGGAG GACACGGGCA  
GGTCCTCTAA TGTTACAGT CCGCGCGCGA CCCCCTCCTC CTGTGCCCGT

11851 GCCTGGAGGC AACCTTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC  
CGGACCTCCG TTGGGATTG ATGGACGACT GGTGGCCGC CGTCTTCTAG

11901 CCCTCGTTGC ACAGTTTAAA CAGCGAGGAG GAGCGCATTT TCGCTACGT  
GGGAGCAACG TGTCAAATTT GTCGCTCCTC CTCGCGTAAA ACGCGATGCA

11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCAGCG  
CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TCGGGTCTCG

12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA  
ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATA CGGAGT

12051 AACCGGCCGT TTATCAACCG CTAATGGAC TACTTGATC GCGCGGCCGC  
TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGCG

12101 CGTGAACCCC GAGTATTTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC  
GCACTTGGGG CTCATAAAGT GGTACGGTA GAACTTGGGC GTGACCGATG

12151 CGCCCCCTGG TTTCTACACC GGGGGATTCTG AGGTGCCCCA GGGTAACGAT  
GCGGGGGACC AAAGATGTGG CCCCCTAAGC TCCACGGGCT CCCATTGCTA

12201 GGATTCTCTT GGGACGACAT AGACGACAGC GTGTTTTCCC CGCAACCGCA  
CCTAAGGAGA CCCTGCTGTA TCTGCTGTCTG CACAAAAGG GCGTTGGCGT

Figure 27 M

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12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC
      TCCTTTTCGAA GCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTCAG ATGCTAGTAG CCCATTCCA AGCTTGATAG GGTCTCTTAC
      GCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCTGCT GGGCGAGGAG GAGTACCTAA
      GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATT
      TGTGAGCGA CGACGTCCGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501 CCCAACAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC
      GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC
      CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG
      CAGTTTCCGT GCTGGCAGTC GCCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCTT GGATTGGA GGGAGTGGCA ACCCGTTTGC
      CGTCTGCTGT CGTCGCAGGA CCTAAACCT CCCTCACCCT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAACAAAAA AAAAAGCATG
      CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTCT
      TACGTTTTAT TTTTGTAGTG GTTCCGTAC CGTGGCTCGC AACCAAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGCG ATGTATGAGG AAGGTCCTCC
      ACATAAGGGG AATCATACGC CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG
      AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTACCCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC
      CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC
      GACGCCGAT GGCCCCCTC TTTGTCGTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG
      GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTC AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC
      ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA
      TAAGTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTGCGACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA
      AGAACTGCTG GCCAGCGTGA CCCC GCCGCT GGACTTTTGG TAGGACGTAT

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Figure 27N

13251 CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA  
GCCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT

13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCGA GGGCAACTAC TCCGAGACCA  
TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT

13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG  
ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTTAC

13401 GGCAGACAGA ACGGGGTTCT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC  
CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACCTGT

13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG  
GGCGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC

13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA  
CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAAGTAAAA CGACGGTCTT

13551 TGCGGGGTGG ACTTCACCCA CAGCCGCGCTG AGCAACTTGT TGGGCATCCG  
ACGCCCCACC TGAAGTGGGT GTCGCGCGAC TCGTTGAACA ACCCGTAGGC

13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG  
GTTGCGCGTT GGGAAGGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC

13651 AGGGTGGTAA CATTCGCGCA CTGTTGGATG TGGACGCCTA CCAGGCGAGC  
TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG

13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GGCGCAGGCG GCAGCAACAG  
AATTTCCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC

13751 CAGTGGCAGC GGCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC  
GTCAACGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTTACG

13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC  
TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG

13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC  
TGTGCCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG

13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA  
GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT

13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC  
AGTTTGGGGA CTGTCTCCTG TCGTTCTTTG CGTCAATGTT GGATTATTG

14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAATA  
TTACTGTCGT GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT

14051 CGGCGACCCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACCTCCTG  
GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC

14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTGCTTGCC AGACATGATG  
TGCAATGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC  
CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251 AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG  
TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCAC  
AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CGCGCGGGCG GTCGGGGGTG

14351 CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC  
GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGCTTA GTGCCCTGCG

14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC  
ATGGCGACGC GTTGTCGTAG CCTCCTCAGG TCGCTCACTG GTAATGACTG

14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC  
CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA  
CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT

14551 TATCGCCAG CAATAACACA GGCTGGGGCC TGCGCTTCCC AAGCAAGATG  
ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG  
AAACCGCCCC GGTTCCTCGC GAGGCTGGTT GTGGGTCACG CGCACGCGCC

14651 GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA  
CGTGATGGCG CGCGGGACCC CGCGCGTGTT TGCGCCGGCG TGACCCGCGT

14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC  
GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751 ACGCCACGC CGCCACCACT GTCCACAGTG GACGCGGCCA TTCAGACCGT  
TGCGGGTGCG GCGGTGGTCA CAGGTGTAC CTGCGCCGGT AAGTCTGGCA

14801 GGTGCGCGGA GCGCGGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG  
CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851 TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCCGCCCA ACGCGCGGCG  
ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TGCGCGCCGC

14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG  
CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA  
CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT

15001 GCGGACGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG  
CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAATCACG ATACTGAGTC

15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG  
CCAGCGTCCC CGTTGCACAT AACCACGCG CTGAGCCAAT CGCCGACGC

Figure 27P

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA  
TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC  
CGATACAGGT TCGCGTTTTA GTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA  
CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGCTT

15301 AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAACCTTGAC  
TCGATTTTCG CAGTTTTC TTTTCTTTC TACTACTACT ACTTGAAC TG

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG  
CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTAC

15401 GAAAGGTGCA CGCGTAAAC GTGTTTTGCG ACCCGGCACC ACCGTAGTCT  
CTTTCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451 TTACGCCCGG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG  
AATGCGGGCC ACTCGCGAGG TGGCGGTGGA TGTTGCGCGA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA  
CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTGCTCG CGGAGCCCCT

15551 GTTTCCTTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG  
CAAACGGATG CCTTTCGCCG TATTCCTGTA CGACCGCAAC GGCAGCTGC

15601 AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG  
TCCCCTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCCTAAAGC GCGAGTCTGG  
GGGCGCGAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTTC CGCTCAGACC

15701 TGACTTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG  
ACTGAACCGT GGGTGGCAGC TCGACTACCA TGGGTTGCGG GTCGCTGACC

15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC  
TTCTACAGAA CCTTTTTC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGCGGC CAATCAAGCA GGTGGCGCCG GGAAGTGGCG TGCAGACCGT  
GCGCACGCCG GTTAGTTCGT CCACCGCGGC CCTGACCCGC ACGTCTGGCA

15851 GGACGTTTCAG ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG  
CCTGCAAGTC TATGGGTGAT GGTATCGTG GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAACG TCCCCGTTG CTCAGCGGT GGC GGATGCC  
TCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCTACGG

15951 GCGGTGCAGG CGGTCGCTGC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA  
CGCCACGTCC GCCAGCGACG CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGCGC CCGCGCCGT  
TTCCCTGGGC ACCTACAAAG CGCAAAGTCG GGGGGCCGCG GGC GCGCAA

Figure 27Q



16051 CGAGGAAGTA CGGCGCCGCC AGCGCGCTAC TGCCCGAATA TGCCCTACAT  
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG  
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC  
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT  
CAGCGGCAGC GGTGCGGCAC GACCGGGGCT AAAGGCACGC GTCCACCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCAG  
GCGCTTCCTC CGTCTGGGA CCACGACGGT TGTCGCGCA TGGTGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT  
GTAGCAAAT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG  
CGCGGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGACCA  
TCCCCGTACC GGCCGGTGCC GGA CTGCCCCG CCGTACGCAG CACGCGTGST

16451 CGGCGGGCGG CGCGCGTCGC ACCGTGCGAT GCGCGGCGGT ATCCTGCCCC  
GGCCGCCGCC GCGCGCAGCG TGGCAGCGTA CGCGCCGCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA  
AGGAATAAGG TGA CTAGCGG CGCCGCTAAC CGCGGCACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTAAAAACAA GTTGCAATGTG  
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTGTG CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCTGTAA  
CTTTTAGTT TTATTTTCA GACCTGAGAG TCGGAGCGAA CCAGGACATT

16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCC GCAGCA  
GATAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA  
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT  
ACTCGCCACC GCGGAAGTCG ACCCCGAGCG ACACCTCGCC GTAATTTTAA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC  
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTC CAACAAAAGG  
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTTAAAG GTTGTTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGA CCTGGCCAAC  
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCCT  
GTCCGTCACG TTTTATTCTA ATTGTCATT GAACTAGGGG CGGGAGGGCA

Figure 27R

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC  
TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCATAAAG CAAGGCCTGC CCACCACCCG  
CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGGC

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA  
AGGGTAGCGC GGGTACCGAT GGCCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCC GCGACACCC AGCAGAAACC TGTGCTGCCA  
GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGCCCGACCG CCGTTGTTGT AACCCGTCTT AGCCGCGCGT CCCTGCGCCG  
CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGGC

17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC  
GCGGCGGTG CAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC  
TTTCGTGTGA CTTGTCGTAG CACCCAGACC CCCACGTTAG GGACTTCGCG

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC  
GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG

17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG  
GTACAGCGGC GGTCTCCTCG ACGACTCGGC GGC GCGCGGG CGAAAGGTTT

17501 ATGGCTACCC CTTCGATGAT GCCGCAGTGG TCTTACATGC ACATCTCGGG  
TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGCAG TTTGCCCCGG  
GGTCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AACCGGGCGC

17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG  
GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACGACGTGAC CACAGACCGG TCCCAGCGTT TGACGCTGCG  
CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT  
CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCCTAGC TGTGGGTGAT AACCGTGTC TGGACATGGC TTCCACGTAC  
AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC  
AAACTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG  
ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAA TAAACCTAGA AGAAGAGGAC  
TTACCCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCCTG

Figure 275

17951 GATGACAACG AAGACGAAGT AGACGAGCAA GCTGAGCAGC AAAAACTCA  
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTGTGAGT

18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA  
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCTCCCAT

18051 TTCAAATAGG TGTCGAAGGT CAAACACCTA AATATGCCGA TAAAACATTT  
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTGTAAAA

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA  
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAAGAC TACCCCAATG AAACCATGTT  
AGTACGTCGA CCCTCTCAGG ATTTTCTCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTTATA TGCAAAACCC ACAATGAAA ATGGAGGGCA AGGCATTCTT  
TGCCAAGTAT ACGTTTGGG TGTCTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGAAA TGCAATTTTT  
CATTTCTGTTG TTTTACCTTT CGATCTTCA GTTCACCTTT ACGTTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG  
GAGTTGATGA CTCCGTCGGC GTCCGTACC ACTATTGAAC TGAGGATTTC

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGA CACTCATATT  
ACCATAACAT GTCACCTCTA CATCTATATC TTTGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA  
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA  
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC  
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTTGTAGAT TTGCAAGACA GAAACACAGA  
GTTCTAGTACG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT  
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT  
AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA

18701 ATTGAAAATC ATGGAACCTGA AGATGAACCT CCAAATTACT GCTTCCACT  
TAACCTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAAACAG  
CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTG

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAT  
CAGTCCTTTT ACCTACCCTT TTTCTACGAT GTCTTAAAG TCTATTTTAA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA  
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC  
TCGATTTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA  
ATGCTGATGT ACTTGTTTCG TCACCACCGA GGGCCCCGATC ACCTGACGAT

19051 CATTAACCTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC  
GTAATTGGAA CCTCGTGCGA CCAGGGAAC TATATACCTG TTGCAGTTGG

19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG  
GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT  
CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA  
ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251 ACTTCAGGAA GGATGTTAAC ATGGTCTGTC AGAGCTCCCT AGGAAATGAC  
TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301 CTAAGGGTTG ACGGAGCCAG CATTAGTTT GATAGCATTT GCCTTTACGC  
GATTCCCAAC TGCCTCGGTC GTAATTCAA CTATCGTAAA CGGAAATGCG

19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC  
GTGGAAGAAG GGGTACCGGG TGTGTGGCG GAGGTGCGAA CTCCGGTACG

19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC  
AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCCG

19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT  
TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTGTCACG GGTATAGGTA

19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCTTC ACGCGCCTTA  
GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC  
TCTGATTCTT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC  
ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651 CTTTAAGAAG GTGGCCATTA CCTTTGACTC TTCTGTCAGC TGGCCTGGCA  
GAAATTCTTC CACCGGTAAT GGAACTGAG AAGACAGTCG ACCGGACCGT

19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC  
TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTCGC GAGTCAACTG

19751 GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCTT  
CCCCCCCAA TGTGCAACG GGTCACATTG TACTGGTTTC TGACCAAGGA

19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC  
CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCCAGCCC  
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGGA TGATACTAAA TACAAGGACT ACCAACAGGT  
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTCTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC  
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT  
GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGGCGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA  
TATCCGTTCT GCGCTCAACT GTCGTAATGG GTCTTTTCA AAGAAACGCT

20101 TCGCACCCCTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG  
AGCGTGGGAA ACCGCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCCACGCG  
GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TCGGGTTGAG GCGGGTGCGC

20201 CTAGACATGA CTTTTGAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA  
GATCTGTACT GAAAACCTCA CCTAGGGTAC CTGCTCGGGT GGGAAGAAAT

20251 TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG  
ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GCGGTGGCGC

20301 GCGTCATCGA AACCGTGAC CTGCGCACGC CTTTCTCGGC CGGCAACGCC  
CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC  
TGTTGTATTT CTTCTGTTCTG TGTAGTTGTT GTCGACGCGC GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAA GATCTTGTT GTGGGCCATA  
TCACTCGTCC TTGACTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA  
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA  
TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CCTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT  
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTTG  
ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCAGCCGC  
TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC  
ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCCTT GCCAACTGGC  
CGGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27 V.

20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TCGCTCGCAA  
GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA  
GGTCCTTGTC GAGATGTCTA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTGTCTA CTTGAAAAAC  
CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA  
TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAAT

21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCTTGCC GTCTGCGCCG  
AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGG

21101 TTTAAAAATC AAAGGGGTTC TGCCGCGCAT CGCTATGCGC CACTGGCAGG  
AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC

21151 GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC  
CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG

21201 CATCCGCGGC AGCTCGGTGA AGTTTTCCTT CCACAGGCTG CGCACCATCA  
GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251 CCAACGCGTT TAGCAGGTCG GCGCCGATA TCTTGAAGTC GCAGTTGGGG  
GGTTGCGCAA ATCGTCCAGC CCGCGGTAT AGAACTTCAG CGTCAACCCC

21301 CCTCCGCCCT GCGCGCGCGA GTTGCGATAC ACAGGGTTGC AGCACTGGAA  
GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTGCGAGA  
GTGATAGTCG CGGCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC  
AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG

21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCAGGCT TTGAGTTGCA  
AAACCATCGA CGGAAGGGTT TTTCCGCGC ACGGGTCCGA AACTCAACGT

21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCCTG TGGGCGTTAG  
GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC  
CTATGTCGCG GACGTATTTT CGGAAGTAGA CGAATTTTCG GTGGACTCGG

21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT  
AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA

21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTGCGTCG GTGTTGGAGA  
CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT

21701 TCTGCACCAC ATTTGCGCCC CACCGGTTCT TCACGATCTT GGCCTTGCTA  
AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT  
TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA

21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC  
GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTGCGCGT CGGGCACCCG

21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG  
AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TCGGACGTC

21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT  
CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA

22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGACATAC GGCCGCCAGA  
CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT

22051 GCTTCCACTT GGTGAGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC  
CGAAGGTGAA CCAGTCCGTC ATCAAACCTC AAGCGGAAAT CTAGCAATAG

22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC  
GTGCACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGAAGAGGG

22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT  
TGCGTCTGTG CTAGCCGTGT GAGTCGCCCCA AGTAGTGGCA TTAAAGTGAA

22201 TCCGCTTCGC TGGGCTCTTC CTCTTCCTCT TCGCTCCGCA TACCACGCGC  
AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGCGCG

22251 CACTGGGTGCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC  
GTGACCCAGC AGAAGTAAGT CGGCGCGCTG ACACGCGAAT GGAGGAAACG

22301 CATGCTTGAT TAGCACCGGT GGGTTGCTGA AACCACCAT TTGTAGCGCC  
GTACGAACTA ATCGTGGCCA CCCAACGACT TTGGGTGGTA AACATCGCGG

22351 ACATCTTCTC TTTCTTCCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG  
TGTAAGAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC

22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGCAATGG  
CGCGAGCCCG AACCCTCTTC CCGCAAGAA AAAGAAGAAC CCGCTTACC

22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC  
GGTTTAGGCG GCGGCTCCAG CTACCGCGC CCGACCCACA CGCGCCGTGG

22501 AGCGCGTCTT GTGATGAGTC TTCTCGTCC TCGGACTCGA TACGCCGCCT  
TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCTGAGCT ATGCGGCGGA

22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGCGGCGAC GGGGACGGGG  
GTAGGCGAAA AAACCCCGC GGGCCCTCC GCCGCGCTG CCCCTGCCCC

22601 ACGACACGTC CTCCATGGTT GGGGACGTC GCGCCGCACC GCGTCCGCGC  
TGCTGTGAG GAGGTACCAA CCCCCTGCAG CGCGCGGTGG CGCAGGCGCG

22651 TCGGGGGTGG TTTCGCGCTG CTCCTCTTCC CGACTGGCCA TTCTCTTCTC  
AGCCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27 X

22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG  
GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCGG CTTGAGGAGG AGGAAGTGAT  
GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG  
ATAGCTCGTC CTGGGTCCAA AACATTGCT TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG  
ATGGTTGTCT CCTATTTTTT GTTCTGGTCC TGTGCGTCT CCGTTTGCTC

22951 GAACAAGTCG GCGGGGGGGA CGAAAGGCAT GCGGACTACC TAGATGTGGG  
CTTGTTTCAGC CCGCCCCCTC GCTTTCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG  
TCTGCTGCAC GACAACTTCG TAGACGTCGC GGTCACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC  
TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCTACG AACGCCACCT ATTCTACCG CGCGTACCCC CCAAACGCCA  
GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTTGCGGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT  
TCTTTTGCCG TGTACGCTCG GGTGGGGCG GGAGTTGAAG ATGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAACCTGC  
AACGGCACGG TCTCCACGAA CCGTGGATAG TGTAGAAAAA GGTTTTGACG

23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT  
TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTGCTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG  
CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC  
ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTTGGT  
CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAACTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG  
CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTCAACCA CTTTGCTTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG  
TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCTGGAGAG  
TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG  
CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y



23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA  
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG  
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCTGCAAG  
TTTGTAACGT GATGTGGAAG GCTGTCCCGA TGCATGCGGT CCGGACGTTT

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA  
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG  
GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951 CGCGCCCGCA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC  
GCGCGGCGCT GATGCAGGCG CTGACGCAAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGAGG AGTGCAACCT  
ACCGTCTGCC GGTACCCGCA AACCCTCGTC ACGAACCTCC TCACGTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG  
GTTCTCGAC GTCTTTGACG ATTTCTGTTT GAACTTCCTG GATACCTGCC

24101 CTTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC  
GGAAGTTGCT CGCGAGGCAC CGCGCGGTGG ACCGCTGTA GTAAAAGGGG

24151 GAACGCCTGC TTAAAACCTT GCAACAGGGT CTGCCAGACT TCACCAGTCA  
CTTGCGGACG AATTTTGGGA CGTTGTCCCA GACGGTCTGA AGTGGTCACT

24201 AAGCATGTTG CAGAACTTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT  
TTCGTACAAC GTCTTGAAAT CTTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCCGCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAACTAC  
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC  
GCGCTTACGG GAGGCGGCGA AACCCTCGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG  
GTTGATGGAA CGGATGGTGA GACTGTATTA CTTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC  
CAGATGACCT CACAGTGACA GCGACGTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTCGCAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT  
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTGGA  
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT  
TTGAGTGAGG CCCCAGACCC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 272

24601 GAGGACTACC ACGCCCACGA GATTAGGTTT TACGAAGACC AATCCCGCCC  
 CTCCTGATGG TCGGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG  
 24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG  
 CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC  
 24701 GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG  
 CGGTAAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC  
 24751 GGACGGGGGG TTTACTTGGA CCCCCAGTCC GCGGAGGAGC TCAACCCAAT  
 CCTGCCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA  
 24801 CCCCCGCGC CCGCAGCCCT ATCAGCAGCA GCCGCGGGCC CTTGCTTCCC  
 GGGGGGCGGC GCGGTCGGGA TAGTCGTCTG CCGCGCCCCG GAACGAAGGG  
 24851 AGGATGGCAC CCAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA  
 TCCTACCGTG GGTTTTCTT CGACGTCGAC GCGGCGGGTG GGTGCTGCT  
 24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGTTTTGGAC GAGGAGGAGG  
 CCTCCTTATG ACCCTGTCTAG TCCGTCTCCT CCAAACCTG CTCCTCCTCC  
 24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC  
 TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG  
 25001 GAAGAGGTGT CAGACGAAAC ACCGTACCCC TCGGTGCGAT TCCCCTCGCC  
 CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG  
 25051 GGCGCCCCAG AAATCGGCAA CCGGTTCCAG CATGGCTACA ACCTCCGCTC  
 CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG  
 25101 CTCAGGCGCC GCCGGCACTG CCCGTTCCGC GACCCAACCG TAGATGGGAC  
 GAGTCCGCGG CGGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG  
 25151 ACCACTGGAA CCAGGGCCCG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA  
 TGGTGACCTT GGTCCCGGCC ATTCAGGTTT GTCGGCGGCG GCAATCGGGT  
 25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAACG  
 TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTCTTGC  
 25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTGCCCCGC  
 GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG  
 25301 CGCTTTCTTC TCTACCATCA CGGCGTGGCC TTCCCCGTA ACATCCTGCA  
 GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT GTAGGACGT  
 25351 TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA  
 AATGATGGCA GTAGAGATGT CGGGTATGAC GTGGCCGCGG TCGCCGTCGT  
 25401 ACAGCAGCGG CCACACAGAA GCAAAGCGGA CCGGATAGCA AGACTCTGAC  
 TGTCGTGCGC GGTGTGTCTT CGTTTCCGCT GGCCTATCGT TCTGAGACTG  
 25451 AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC  
 TTTCGGGTTC TTTAGGTGTC GCCGCCGTCG TCGTCTCTCT CCTCGCGACG  
 25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT  
 CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCTTAA

Figure 27 AA

25551 TTTCCCACTC TGTATGCTAT ATTTCAACAG AGCAGGGGCC AAGAACAAGA  
 AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTCT

25601 GCTGAAAATA AAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT  
 CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651 ATCACAAAAG CGAAGATCAG CTTGCGGCGA CGCTGGAAGA CGCGGAGGCT  
 TAGTGTTTTC GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT  
 GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCCTGATCA AAGCGCGGGA

25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG  
 AAGAGTTTAA ATTGCGGCTT TTGATGCAGT AGAGGTCGCC GGTGTGGGCC

25801 CGCCAGCACC TGTTGTCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC  
 GCGGTCGTGG ACAACAGTCG CGGTAATACT CGTTCCTTTA AGGGTGCGGG

25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCA  
 ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT

25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT  
 TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA

25951 CCCGGGTCAA CGGAATACGC GCCCACCAGG ACCGAATTCT CCTGGAACAG  
 GGGCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC

26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC  
 CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG

26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC  
 GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAACTCAGG GGCGCAGCTT  
 GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151 GCGGGCGGCT TTCGTACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA  
 CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT

26201 CCTGACAATC AGAGGGCGAG GTATTGAGCT CAACGACGAG TCGGTGAGCT  
 GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC  
 GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG

26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAACCTCTG AGACCTCGTC  
 GCGAGAAGTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT  
 GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA

26401 TTGTGCCATC GGTCTACTTT AACCCTTCT CGGGACCTCC CGGCCACTAT  
 AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGGAGG GCCGGTGATA

26451 CCGGATCAAT TTATTCTTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG  
 GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTTCTTGA GCCGCCTGCC

Figure 27 AB

26501 CTACGACTGA ATGTTAAGTG GAGAGGCAGA GCAACTGCGC CTGAAACACC  
GATGCTGACT TACAATTCAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT  
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCGA GGATCATATC GAGGGCCCCG CGCACGGCGT  
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCG TAGCCTGATT CGGGAGTTTA  
GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTTCCTACT  
GGGTGCGGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCCTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA  
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAATATA CTGGGGCTCC  
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG  
ATAGCGGTAG GACATTTGCG GTGGCAGAAG TGGGCGGGTT CGTTTGGTTC

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA  
CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTCAACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC  
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG  
ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGGCCC TGGCATGCTC

27051 TGCCTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT  
ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT  
AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA  
ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA  
ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTG TG ATTCTCTTTA TTCTTATACT  
TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCTG CTGTGTGCAC ATTTGCATTT  
TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA  
TAACAGTCGA AAAATTGCG ACCCCAGCGG TGGGTTCTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTGCGTCAG CCCACGGTAC CACCCAAAAG  
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTT

Figure 27AC

27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTCCGAG CTGAAGCTAA  
CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA  
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG  
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA  
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA  
ATTTTGAAAA TACATATGAA AAGGTAAAA ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCACAAAA TTGTGTGGAA  
ACATGTACTC GTTGTCTATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT  
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTA CTCTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG  
CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC  
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT  
ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATT  
TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA  
GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAAC

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG  
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA  
GCCTAAACAA GGTGAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAAACAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA  
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG  
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT  
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT  
CGACGGATTT CCGGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGAATCCAT AGATTGGACG GACTGAAACA  
CACGATGTGG GTTGTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG  
 AAAATATAAT GACTGGGAAC AACGCCAAAA AACACGCACG AGGTGTAACC  
 28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT  
 GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA  
 28551 TTGCTTTACG GATTTGTCAC CCTCACGCTC ATCTGCAGCC TCATCACTGT  
 AACGAAATGC CTAAACAGTG GGAGTGCGAG TAGACGTCGG AGTAGTGACA  
 28601 GGTCAATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT  
 CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA  
 28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT  
 TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA  
 28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTCTGCT GATTATTTGC  
 TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG  
 28751 ACCCTATCTG CGTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC  
 TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG  
 28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAAA  
 TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACGATG TTACTTTTTT  
 28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC  
 CGCTAGAAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG  
 28901 TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG  
 ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC  
 28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC  
 CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG  
 29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT  
 AAGGTGACGT TGTTCACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA  
 29051 CGCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG  
 GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC  
 29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG  
 TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC  
 29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA  
 GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT  
 29201 TCAAGAGCTC CAAGACATGG TTAACCTGCA CCAGTGCAAA AGGGGTATCT  
 AGTTCCTGAG GTTCTGTACC AATTGAACGT GGTACGTTT TCCCCATAGA  
 29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA  
 AAACAGAGCA TTTCGTCCGG TTTCAGTGGA TGCTGTCATT ATGGTGGCCT  
 29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT  
 GTGGCGGAAT CGATGTTCAA CGGTTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27AE

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT  
CGACGTAAGT GAGTGGAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA  
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501 AATAATAAAG CATCACTTAC TTAAATCAG TTAGCAAAT TCTGTCCAGT  
TTATTATTTT GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT  
AATAAGTCGT CGTGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT  
GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCTCG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG  
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC  
GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAACG TGCCTTTTCT TACTCCTCCC TTTGTATCCC  
CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCCTGGGG TACTCTCTTT GCGCCTATCC  
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG  
CTTGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAT GTAACCACTG  
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951 TGAGCCCAAC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT  
ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC  
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GCGGGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA  
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGCACGA CTCCAACTT AGCATTGCCA CCAAGGACC CCTCACAGTG  
GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA  
AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG  
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA  
CATCGAACCC GTAACGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC  
AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAACATAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA  
TTTGATTTC AATGACCTCGG AACCCAAAAC TAAGTGTTC GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT  
GAATTACATC GTCCTCCTGA TTCCTAATA AGAGTTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC  
TGAATACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCCTCTTTTT ATAACTCAG CCCACAACCT GGATATTAAC  
ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTACAGCT TCAAAACAATT CCAAAAAGCT  
ATGTTGTTTC CGGAAATGAA CAAATGTCGA AGTTTGTTAA GGTTTTTCGA

30651 TGAGGTAAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA  
ACTCCAATTG GATTCGTGAC GGTTCCTCAA CTACAACTG CGATGTCGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA  
ATCGGTAATT ACGTCCTCTA CCCGAACCTA AACCAAGTGG ATTACGTGGT

30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTG  
TTGTGTTTAG GGGAGTTTTG TTTTAAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA  
TTTGTTCGGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACGTGTCG

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG  
GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTGTA TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC  
TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTACGTC TCTTCTACG

30951 TAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG  
ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTGAGTTTT GGCTGTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT  
AAAGTCAAAA CCGACAATTT CCGTCAAAAC GAGGTTATAG ACCTTGTCAA

31051 CAAAGTGCTC ATCTTATTAT AAGATTTGAC GAAAATGGAG TGCTACTAAA  
GTTTCACGAG TAGAATAATA TTCTAACTG CTTTACCTC ACGATGATTT

31101 CAATTCCCTC CTGGACCCAG AATATTGGA CTTTAGAAAT GGAGATCTTA  
GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA  
GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCAGTCA  
CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG



31251 AGTTTACTTA AACGGAGACA AACTAAACC TGTAACACTA ACCATTACAC  
TCAATGAAT TTGCCTCTGT TTTGATTGG ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG  
ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTACG TATGAGATA

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC  
AGTAAAAGTA CCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTTT CATACTTGC CCAAGAATAA AGAATCGTTT  
GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTCAA TTGCAGAAAA TTTCAAGTCA  
CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501 TTTTTCATTC AGTAGTATAG CCCACCACC ACATAGCTTA TACAGATCAC  
AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACTCAC AGAACCTAG TATTCACCT GCCACCTCCC  
GCATGGAATT AGTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCC GGCTGG CCTTAAAAAG  
AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTT

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG  
GTAGTATAGT ACCCATGTG TGTATAAGAA TCCACAATAT AAGGTGTGCG

31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC  
AAAGGACAGC TCGGTTTGGC AGTAGTCACT ATAATTATTT GAGGGGCCCCG

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG  
TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801 TCCAACTTGC GGTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA  
AGGTTGAACG CCAACGAATT GCGGCCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC  
ACCCCATCT CAGTATTAGC ACGTAGTCCT ATCCGCCCAC CACGACGTG

31901 AGCGCGCGAA TAACTGCTG CCGCCGCCGC TCCGTCCTGC AGGAATACAA  
TCGCGCGCTT ATTTGACGAC GCGCGCGCG AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCCTCAG CGATGATTG CACCGCCCGC AGCATAAGGC  
GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCG

32001 GCCTTGTCCT CCGGGCACAG CAGCGCAGCC TGATCTCACT TAAATCAGCA  
CGGAACAGGA GGCCCGTGTC GTCGCGTGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAATCC CACAGTGCAA  
GTCAATTGACG TCGTGTCTG GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GGCCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAAGCC ACGTGGCCAT  
CCGCGACATA GGTTCGAGT ACCGCCCTG GTGTCTTGGG TGCACCGTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG  
GTATGGTGT CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC  
GGTATATTG GAGACTAATT TGTACC GCGG TAGGTGGTGG TAGGATTTGG

32301 AGCTGGCCAA AACCTGCCCCG CCGGCTATAC ACTGCAGGGA ACCGGGACTG  
TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC

32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT  
CTTGTTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA

32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC  
GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG

32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGGAAACAACC  
AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTTGG

32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA  
GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT

32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT  
TGAGTGCAAC ACGTAAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA

32601 CCTCCAGTAT GG TAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC  
GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTCCTCC ATCTGCTAGG

32651 CTACTGTACG GAGTGC GCGG AGACAACCGA GATCGTGTG GTCGTAGTGT  
GATGACATGC CTCACGCGGC TCTGTGGCT CTAGCACAAAC CAGCATCACA

32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTGAA GCAAAAACCG  
GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC

32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG  
CACGCCCCA CTGTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC

32801 CTCTGTGTAG TAGTTGTAGT ATATCCAATC TCTCAAAGCA TCCAGGCGCC  
GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG

32851 CCCTGGCTTC GGGTTCTATG TAAACTCCTT CATGCGCCGC TGCCCTGATA  
GGGACCGAAG CCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT

32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTTCGTT  
TGTAGGTGGT GCGTCTTAT TCGGTGTGGG TCGGTTGGAT GTGTAAGCAA

32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT  
GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA

33001 TTTTTTTATT CAAAAGATT ATCCAAAACC TCAAATGAA GATCTATTAA  
AAAAAATAA GGTTTCTAA TAGGTTTGG AGTTTACTT CTAGATAATT

33051 GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAATCTACA GCCAAAGAAC  
CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTCTTG

33101 AGATAATGGC ATTTGTAAGA TGTGCACAA TGGCTTCCAA AAGGCAAACG  
TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC  
GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG

33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCGGGCC  
CGGTGGAAGA GTTATATAGA GATTCGTTTA GGGCTTATAA TTCAGGCCGG

33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG  
TAACATTTTT AGACGAGGTC TCGCGGGAGG TGAAGTCGG AGTTCGTCGG

33351 AATCATGATT GCAAAAATTC AGGTTCTCTCA CAGACCTGTA TAAGATTCAA  
T TAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG  
TTCGCCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC  
GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501 CCGCCAGGAA CCATGACAAA AGAACCACACA CTGATTATGA CACGCATACT  
GGCGGTCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG  
GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTCGAACA ACGTACCCGC

33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC  
CGCTATATTT TACGTTCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG

33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG  
TTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATT

33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG  
GAGGCCTTGG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT  
GCCAAAGAC GTATTGTGT TTTATTTTAT TGTTTTTTG TAAATTTGTA

33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG  
ATCTTCGGAC AGAATGTTGT CCTTTTGTG GGAATATTC GTATTCTGCC

33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA  
TGATGCCGGT ACGGCCGCAC TGGCATTITT TTGACCAGTG GCACTAATTT

33901 AAGCACCACC GACAGCTCCT CGGTCATGTC CGGAGTCATA ATGTAAGACT  
TTGCTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951 CGGTAAACAC ATCAGGTGTA TTCACATCGG TCAGTGCTAA AAAGCGACCG  
GCCATTGTG TAGTCCAAC T AAGTGTAGCC AGTCACGATT TTTCGCTGGC

34001 AAATAGCCCG GGGGAATACA TACCCGAGG CGTAGAGACA ACATTACAGC  
TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG

34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC  
GGGGTATCCT CCATATGTT TTAATTATCC TCTCTTTTTG TGTATTTGTG

Figure 27A J

34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA  
 TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT  
 34201 AAAAGAAAAC CTATTAAAAA AACACCACTC GACACGGCAC CAGCTCAATC  
 TTTTCTTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG  
 34251 AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA  
 TCAGTGTCAC ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT  
 34301 AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC  
 TTTTACTGCG ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG  
 34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCTCTAAA  
 CGCTTGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT  
 34401 TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAAAC  
 AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA  
 34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG  
 TGTTAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGGG TGCAGTGGGC  
 34501 CCCC GTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC  
 GGGGCAAGGG TCGGGGGCGC GGTGCACTGT TTGAGGTGGG GGAGTAATAG  
 PacI  
 -----  
 34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG  
 TATAACCGAA GTTAGGTTTT ATTCCATATA ATAACCTACTA CAATTAATTC  
 34601 AATTCGGATC TCGACGCGA GGCTGGATGG CCTTCCCAT TATGATTCTT  
 TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA  
 34651 CTCGCTTCCG GCGGCATCGG GATGCCCGCG TTGCAGGCCA TGCTGTCCAG  
 GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC  
 34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA  
 CGTCCATCTA CTGCTGGTAG TCCCTGTCGA AGTTCCGGTC GTTTTCCGGT  
 34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC  
 CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG  
 34801 CCTGACGAGC ATCACA AAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC  
 GGACTGCTCG TAGTGT TTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG  
 34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC  
 CTGTCTTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG  
 34901 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC  
 CGAGAGGACA AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG  
 34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG  
 GGAAGCCCTT CGCACC CGCA AAGAGTATCG AGTGCGACAT CCATAGAGTC  
 35001 TTCGGTGTAG GTCGTTTCGT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG  
 AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC

Figure 27 AK

AAGTCGGGCT GCGCAGCGCG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT  
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCTTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC  
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACCTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG  
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA  
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC  
TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTCGTC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT  
CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG TCATGAGATT  
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA  
TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
ACTCATTGTA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC  
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCGAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC  
CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGCAATAA  
TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC  
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC  
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG  
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA  
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC  
AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGCCCGCA GTGTTATCAC  
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG

Figure 2 AL

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA  
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAACA CGGGATAATA  
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTTGG AAAACGTTCT  
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTTCGAT  
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA  
CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA  
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTCCTT

36351 ATAAGGGCGA CACGGAATG TTGAATACTC ATACTCTTCC TTTTCAATA  
TATTCCTGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG  
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA  
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAAACCTA  
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA  
ATTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

## PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)  
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27A M

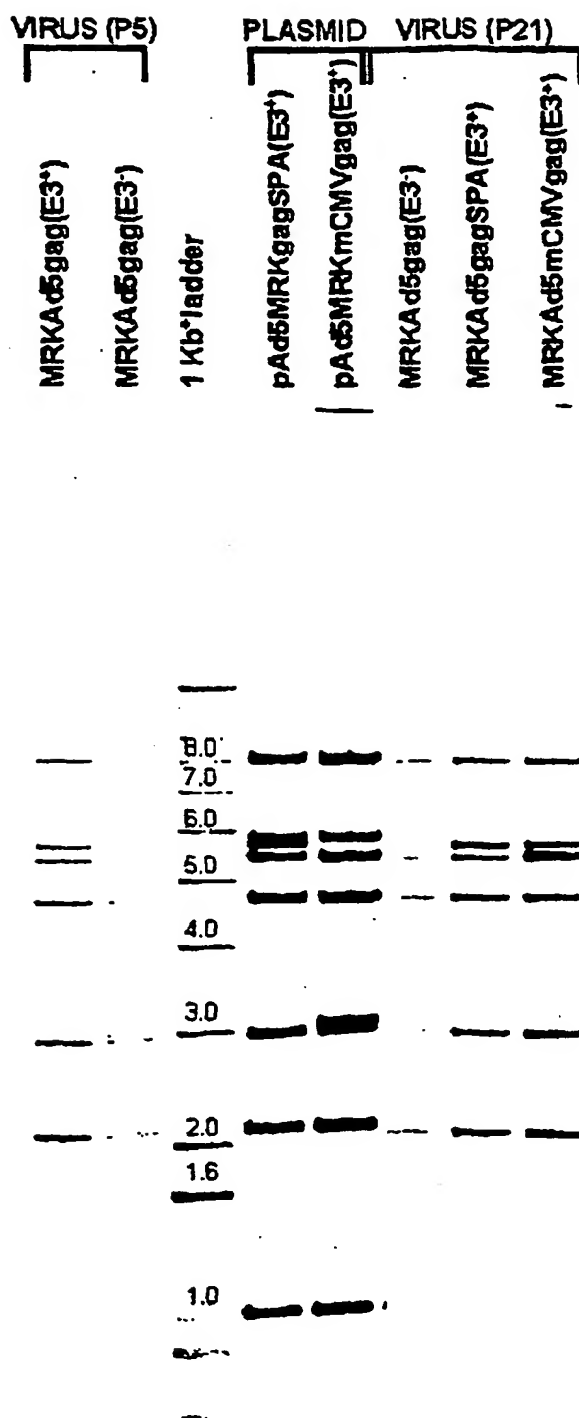


FIGURE 28

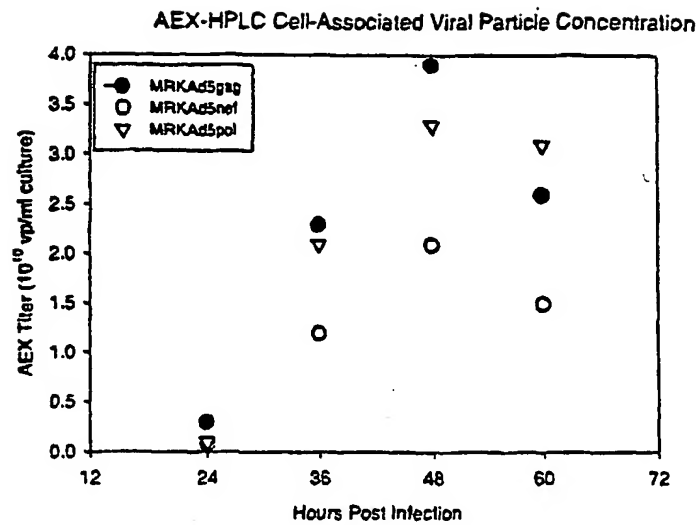


FIGURE 29A

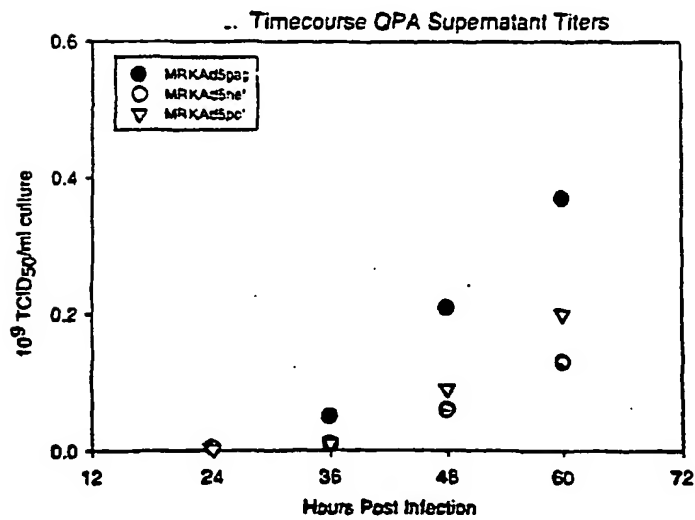


FIGURE 29B



|                                                                 |     |
|-----------------------------------------------------------------|-----|
| atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga | 48  |
| Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly |     |
| 1 5 10 15                                                       |     |
| gca gtc ttc gtt tgc ccc agc gag atc tcc att gtg tgg gcc tcc agg | 96  |
| Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg |     |
| 20 25 30                                                        |     |
| gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag | 144 |
| Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu |     |
| 35 40 45                                                        |     |
| ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc | 192 |
| Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly |     |
| 50 55 60                                                        |     |
| tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt | 240 |
| Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys |     |
| 65 70 75 80                                                     |     |
| gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag | 288 |
| Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys |     |
| 85 90 95                                                        |     |
| att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct | 336 |
| Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala |     |
| 100 105 110                                                     |     |
| gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg | 384 |
| Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val |     |
| 115 120 125                                                     |     |
| cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc | 432 |
| Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr |     |
| 130 135 140                                                     |     |
| ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag | 480 |
| Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu |     |
| 145 150 155 160                                                 |     |
| gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac | 528 |
| Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp |     |
| 165 170 175                                                     |     |
| ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag | 576 |
| Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln |     |
| 180 185 190                                                     |     |
| atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg | 624 |
| Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu |     |
| 195 200 205                                                     |     |
| cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc | 672 |
| His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro |     |
| 210 215 220                                                     |     |
| agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att | 720 |
| Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile |     |
| 225 230 235 240                                                 |     |
| ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag | 768 |
| Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys |     |
| 245 250 255                                                     |     |

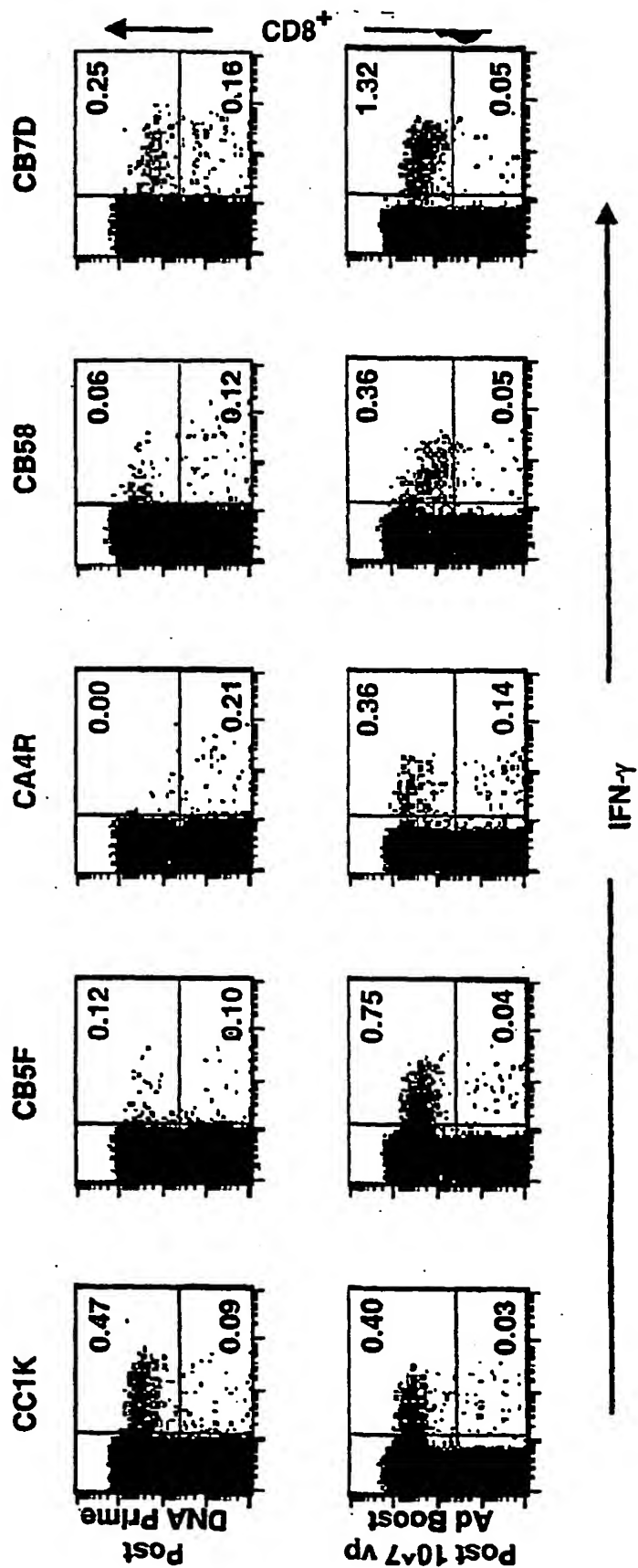
Figure 30A

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc<br>Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro<br>260 265 270     | 816  |
| acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac<br>Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp<br>275 280 285     | 864  |
| tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag<br>Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln<br>290 295 300     | 912  |
| gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac<br>Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn<br>305 310 315 320 | 960  |
| cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg<br>Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu<br>325 330 335     | 1008 |
| gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag<br>Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys<br>340 345 350     | 1056 |
| gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc<br>Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr<br>355 360 365     | 1104 |
| atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag<br>Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys<br>370 375 380     | 1152 |
| tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc<br>Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala<br>385 390 395 400 | 1200 |
| ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg<br>Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met<br>405 410 415     | 1248 |
| aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc<br>Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro<br>420 425 430     | 1296 |
| tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc<br>Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro<br>435 440 445     | 1344 |
| aca gcc cct ccc gag gag tcc ttc agg ttc ggg gag gag aag acc acc<br>Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr<br>450 455 460     | 1392 |
| ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc<br>Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala<br>465 470 475 480 | 1440 |
| tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36)<br>Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37)<br>485 490   | 1482 |

Figure 30 B

**Figure 31**

**IFN- $\gamma$  Secretion against Gag 20-aa pool from CD3<sup>+</sup> T cells of Monkey PBMCs**



# Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost

## Immunizations

Ad Prime/Boost

DNA-CRL1005 Prime/Ad Boost

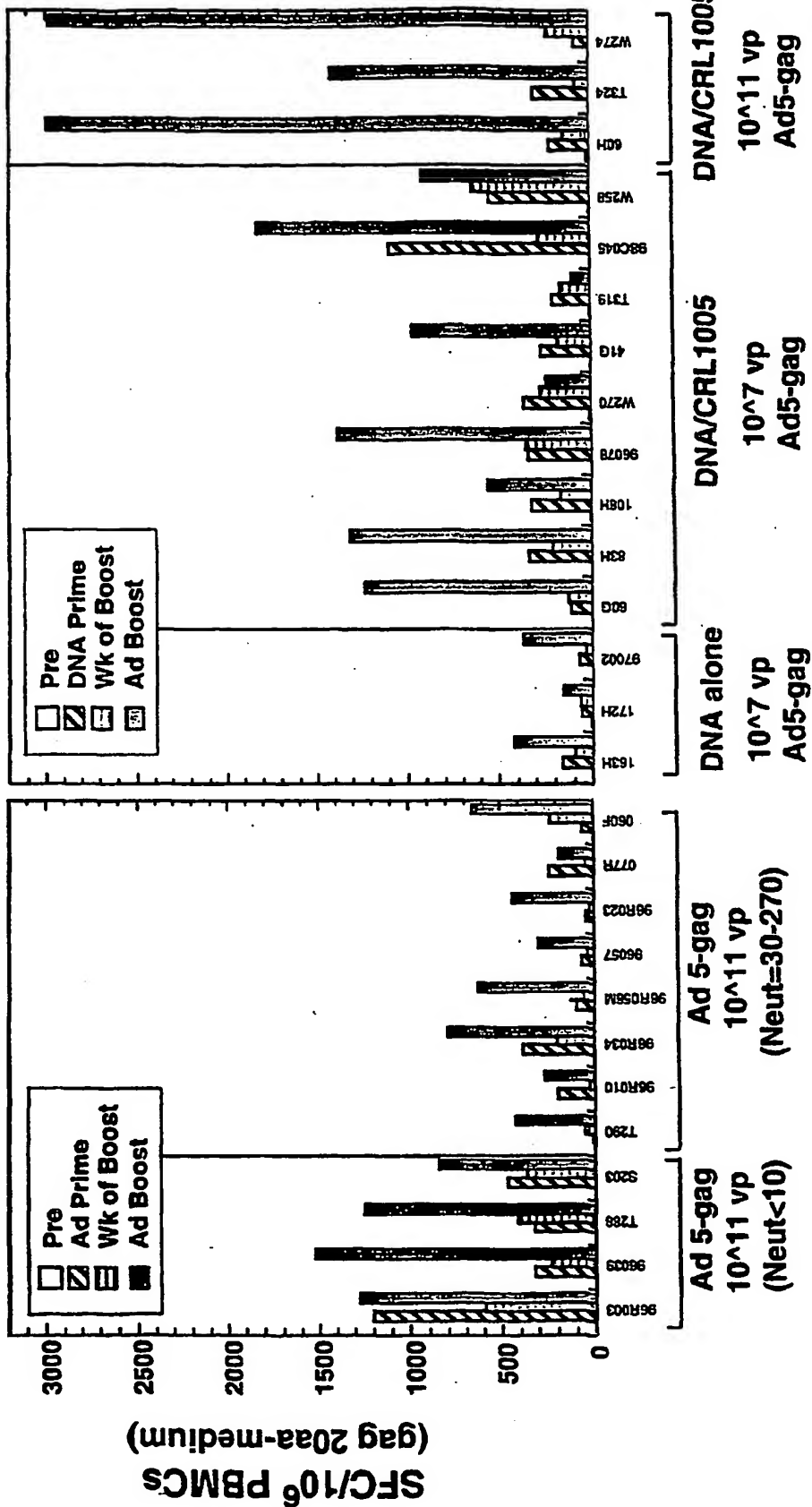


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG  
CTGAGGCTTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG  
CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC  
CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC  
ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC  
CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT  
GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC  
CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG  
GAGAAGGCCT TCTCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC  
CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG  
CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT  
GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC  
TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCATCCC TGTGGGGGAA  
ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCACC  
TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTT  
TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC  
CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT  
GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC  
AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG  
GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC  
ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC  
CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC  
CACAAGGGCA GGCCTGGCAA CTTCCTCCAG TCCAGGCCTG AGCCACAGC CCCTCCCAG  
GAGTCCTTCA GGTTCGGGA GGAGAAGACC ACCCCAGCC AGAAGCAGGA GCCCATTGAC  
AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCAG  
ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC  
CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC  
ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACC CTACAACACC  
CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT  
GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG  
CCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCCTCAT CAACAATGAG  
ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC  
ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC  
AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC  
AAGAAGCACC AGAAGGAGCC CCCCTTCCTG TGGATGGGCT ATGAGCTGCA CCCCAGACA  
TGGACTGTGC AGCCCATTTG GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG  
AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG  
GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC  
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG  
GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG  
GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC  
ACCCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG  
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG  
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CTCCAGGCCA TCTACCTGGC CTTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC  
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG  
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC  
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG  
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC  
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT  
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT  
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC  
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG  
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG  
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG  
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG  
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT  
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACCTC TGACATCAAG  
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT  
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA

SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys  
 Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp  
 Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser  
 Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser  
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln  
 Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln  
 Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser  
 Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys  
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His  
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser  
 Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn  
 Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu  
 Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
 Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr  
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met  
 Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly  
 Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp  
 Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn  
 Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln  
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu  
 Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu  
 Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile  
 Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys  
 Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys  
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr  
 Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu  
 Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu  
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr  
 Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr  
 Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met  
 Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln  
 Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr  
 Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp  
 Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro  
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr  
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu  
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile  
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu  
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr  
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile  
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe  
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile  
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu  
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr  
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp  
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile  
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln  
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu  
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn  
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile  
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val  
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val  
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro  
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp  
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn  
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile  
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val  
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu  
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln  
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu  
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln  
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp  
SEQ ID NO: 39



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Confirmation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category *  | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                              | Relevant to claim No.                                 |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| X<br>—<br>Y | WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.                                                                                                                                          | 1-3, 8-11, 18<br>—<br>4, 5, 13-17, 29-32, 34, 35, 37  |
| X<br>—<br>Y | US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8.                                                                                                                                                             | 1-3, 8-11, 18<br>—<br>4, 5, 13-17, 29-32, 34, 35, 37  |
| X,P         | US 6,287,571 <i>B1</i> (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.                                                                                                                                           | 1, 9, 18                                              |
| X<br>—<br>Y | US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.                                                                                                                                                             | 1-3, 8, 9-11, 18<br>—<br>4,5,13-17, 29-32, 34, 35, 37 |
| Y           | WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683. | 1-3, 9-11, 13-18                                      |



Further documents are listed in the continuation of Box C.



See patent family annex.

| Special categories of cited documents:                                                                                                                                    |                                                                                                                                                                                                                                                    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| * "E" earlier application or patent published on or after the international filing date                                                                                   | * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| * "O" document referring to an oral disclosure, use, exhibition or other means                                                                                            | * "&" document member of the same patent family                                                                                                                                                                                                    |
| * "P" document published prior to the international filing date but later than the priority date claimed                                                                  |                                                                                                                                                                                                                                                    |

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                        | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y          | NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.                                        | 1, 9, 29-32           |
| Y          | PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.                          | 1, 9, 29-32           |
| Y          | LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. | 1, 9                  |
| Y          | PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.                                                                                                    | 16                    |
| Y          | NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.                                                                                                                                     | 1, 9                  |

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## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

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**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

| Group | Claims                                       |                                                                                                                                                                                                                                                                                                                                                                                |
|-------|----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1     | 1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal. |
| 2     | 6, 7, 36                                     | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).                                                                                                     |
| 3     | 12, 33                                       | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.                                                                                          |
| 4     | 19-23, 38-42                                 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.                                                                                                                                                                                                                            |
| 5     | 24, 27, 28, 43, 46, 47                       | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.                                                                                                                                                                                                                               |
| 6     | 25, 26, 44, 45                               | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                                                                                            |
| 7     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.                                                                           |
| 8     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.                                                                           |
| 9     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.                                                                           |
| 10    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.                                                                         |
| 11    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.                                                                         |
| 12    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.                                                                         |
| 13    | 55                                           | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$                                                                                                                                                                                                                                                                                |

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|    |                   |                                                                                                                                                                                                                                                                                                         |
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|    |                   | and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.                                                                                                                  |
| 14 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.                  |
| 15 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.                  |
| 16 | 57-61             | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.                                                                                                                                                     |
| 17 | 62, 65, 66        | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.                                                                                                                                                        |
| 18 | 63, 64            | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                     |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.    |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.   |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.   |
| 22 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.   |
| 23 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.  |
| 24 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1. |
| 25 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1. |
| 26 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1. |
| 27 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.                  |
| 28 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.                 |
| 29 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type                                                                                                                |

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|    |             |                                                                                                                                                                                                                                                                                         |
|----|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |             | adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.                                                                                                                                                                                          |
| 30 | 74          | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. |
| 31 | 76-80       | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.                                                                                                                                     |
| 32 | 81, 84, 85  | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.                                                                                                                                              |
| 33 | 82, 83      | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                           |
| 34 | 86a         | The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.                                                                                                                                                 |
| 35 | 86b, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.                                                                                                                                                 |
| 36 | 86c, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .                                                                            |
| 37 | 86d, 87, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .                                                                            |
| 38 | 86e, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .                                                                            |
| 39 | 86f, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.                                                                                                                                   |
| 40 | 86g, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.                                                                                                                                                              |
| 41 | 86h, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.                                                                                                                                                             |
| 42 | 86i, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.                                                                                                                                                              |
| 43 | 86j, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.                                                                                                                                                        |
| 44 | 86k, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 45 | 86l, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 46 | 86m, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 47 | 86n, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 48 | 86o, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erdi et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter